Class II. **Betaproteobacteria** *class. nov.*

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Be.ta.pro.te.o.bac.te′ ri.a. Gr. n. *beta* name of second letter of Greek alphabet; Gr. n. *Proteus* ocean god able to change shape; Gr. n. *bakterion* a small rod; M.L. fem. pl. n. *Betaproteobacteria* class of bacteria having 16S rRNA gene sequences related to those of the members of the order *Spirillales*.

The class *Betaproteobacteria* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the class contains the orders *Burkholderiales, Hydrogenophilales, Meth-ylophilales, Neisseriales, Nitrosomonadales, "Procabacteriales"*, and *Rhodocyclales*.

Type order: **Burkholderiales** *ord. nov.*

Order I. **Burkholderiales** *ord. nov.*

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Burk.hol.de.ri.a′ les. M.L. fem. n. *Burkholderia* type genus of the order; *-ales* ending to denote order; M.L. fem. n. *Burkholderiales* the *Burkholderia* order.

The order *Burkholderiales* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the order contains the families *Burkholderiaceae, Oxalobacteraceae, Alcaligenaceae*, and *Comamonadaceae*.

Order is phenotypically, metabolically, and ecologically diverse. Includes strictly aerobic and facultatively anaerobic chemoorganotrophs; obligate and facultative chemolithotrophs; nitrogen-fixing organisms; and plant, animal, and human pathogens.

Type genus: **Burkholderia** Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398 (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1268) emend. Gillis, Van, Bardin, Goor, Hebbar, Willems, Segers, Kersters, Heulin and Fernandez 1995, 286.

Family I. **Burkholderiaceae** *fam. nov.*

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Burk.hol.de.ri.a′ ce.ae. M.L. fem. n. *Burkholderia* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Burkholderiaceae* the *Burkholderia* family.

The family *Burkholderiaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the family contains the genera *Burkholderia* (type genus), *Cupriavidus, Lautropia, Limnobacter, Pandoraea, Paucimonas, Polynucleobacter, Ralstonia*, and *Thermothrix. Limnobacter* was proposed after the cutoff date for inclusion in this volume (June 30, 2001) and is not described here (see Spring et al. (2001).

Family is phenotypically, metabolically, and ecologically diverse. Includes both strictly aerobic and facultatively anaerobic chemoorganotrophs and obligate and facultative chemolithotrophs.

Type genus: **Burkholderia** Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398 (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1268) emend. Gillis, Van, Bardin, Goor, Hebbar, Willems, Segers, Kersters, Heulin and Fernandez 1995, 286.

Genus I. **Burkholderia** *Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398*[VP] *(Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1268) emend. Gillis, Van, Bardin, Goor, Hebbar, Willems, Segers, Kersters, Heulin and Fernandez 1995, 286**

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Burk.hol.de′ ri.a. M.L. fem. n. *Burkholderia* named after W.H. Burkholder, American bacteriologist who discovered the etiological agent of onion rot.

*Editorial Note: The literature search for the chapter on *Burkholderia* was completed in January, 2000. During the course of unavoidable publication delays, a number of new species were described or reclassified after the chapter was completed. It was not possible to include these species in the text or to include their characteristics in the comparative tables. The reader is encouraged to consult the studies listed in the Further Reading section.

Cells single or in pairs, **straight or curved rods**, but not helical. Dimensions, generally 0.5–1 × 1.5–4 μm. Motile by means of one or, more commonly, **several polar flagella**. One species (*Burkholderia mallei*) lacks flagella and is nonmotile. Do not produce sheaths or prosthecae. No resting stages are known. **Gram negative.** Most species **accumulate poly-β-hydroxybutyrate (PHB) as**

carbon reserve material. Chemoorganotrophs. Have a strictly **respiratory type of metabolism with oxygen as the terminal electron acceptor. Some species can exhibit anaerobic respiration with nitrate.** Strains of some of the species (*B. cepacia, B. vietnamiensis*) are able to fix N_2. Catalase positive. A wide variety of organic compounds can be used as sources of carbon and energy for growth. Although hydroxylated fatty acids are present in the lipids of members of other genera of aerobic pseudomonads, species of *Burkholderia* are **characterized by the presence of hydroxy fatty acids of 14, 16, and 18 carbon atoms ($C_{14:0\ 3OH}$ and $C_{16:0}$, and $C_{16:0\ 2OH}$, $C_{16:1}$, and $C_{18:1}$). The most characteristic of these acids is the $C_{16:0\ 3OH}$.** Two different ornithine lipids are present in strains of some of the species. Over one-half of the species are **pathogenic for plants or animals (including humans)**. The genus belongs to the ribosomal RNA similarity group II, which can be differentiated from other groups of aerobic pseudomonads by rRNA/DNA hybridization experiments or by rDNA sequencing.

The mol% G + C of the DNA is: 59–69.6.

Type species: **Burkholderia cepacia** (Palleroni and Holmes 1981) Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398 (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1271) (*Pseudomonas cepacia* Palleroni and Holmes 1981, 479.)

FURTHER DESCRIPTIVE INFORMATION

Cell morphology The cells of the genus *Burkholderia* correspond in their general characteristics to those of other aerobic pseudomonads: Gram-negative rods, straight or slightly curved, with rounded ends, usually motile when suspended in liquid. Motility is due to several polar flagella, but a single flagellum per cell has been reported for *B. andropogonis, B. glathei,* and *B. norimbergensis.** The single flagellum of *B. andropogonis* is sheathed (Fuerst and Hayward, 1969a). One species, *B. mallei*, is nonmotile and lacks flagella (Redfearn et al., 1966).

Intracellular granules The cells of species of the genus accumulate granules of carbon reserve material (poly-β-hydroxybutyrate, PHB, which may be part of a copolymer with poly-β-hydroxyvalerate, PHA). Proteins ("phasins") have been found to be associated with the granules (Wieczorek et al., 1996). Only *B. pseudomallei* and some strains of *B. mallei* use extracellular PHB for growth (Ramsay et al., 1990). All species able to accumulate PHB can use the intracellular polymer when needed; however, in the description of some species, the statement "can use PHB" has been included, without indicating whether the PHB was endo- or extracellular.

The colonies of most species of the genus are smooth, but those of the human pathogen *B. pseudomallei* often have a rough surface.

Lipids The first detailed analysis of the fatty acids of aerobic pseudomonads, using both saprophytic and phytopathogenic strains, demonstrated the possibility of establishing a correlation with the phylogenetic subdivision of the genus *Pseudomonas*, as classically defined, on the basis of rRNA–DNA hybridization (Oyaizu and Komagata, 1983). Years later, the results of this survey were confirmed and extended (Stead, 1992). The results of these investigations firmly established the fact that members of

rRNA similarity group II of Palleroni et al. (1973), which includes the genus *Burkholderia*, have a fatty acid composition containing $C_{14:0\ 3OH}$ and $C_{16:0}$, and $C_{18:1\ 2OH}$. Most strains also contained $C_{16:0\ 2OH}$ and $C_{16:1}$. Even though hydroxylated fatty acids are present in the lipids of members of other groups, group II including *Burkholderia* is the only one having hydroxylated fatty acids of 14, 16, and 18 carbon atoms (Table BXII.β.1). A recent evaluation of the taxonomic significance of fatty acid composition emphasizes the diagnostic value of the above results (Vancanneyt et al., 1996), confirming earlier findings on this approach for the characterization of major phylogenetic groups within the pseudomonads (Wollenweber and Rietschel, 1990).

Ornithine-containing lipids in *Pseudomonas aeruginosa, P. putida,* and *B. cepacia* represent from 2–15% of the total of extractable lipids. The amino acid was not found in the phospholipids that amount to more than 80% of all the extractable lipids (Kawai et al., 1988). An analysis of the polar lipids and fatty acids of *B. cepacia* has shown that the only significant phospholipids in this species are phosphatidyl-ethanolamine and bis(phosphatidyl)-glycerol. These characteristics, taken together with the unusual lipid profiles of *B. cepacia*, can be used as markers of chemotaxonomic importance (Cox and Wilkinson, 1989a).

A striking feature of the cellular composition of *B. cepacia* is the range of polar lipids, which include two forms (with and without 2-OH fatty acids) of phosphatidyl-ethanolamine and ornithine amide lipids. Variations in the lipid composition, as well as in pigmentation and flagellation, were observed as the consequence of changes in growth temperature and limiting oxygen, carbon, phosphorus, and magnesium supplies in the medium. Phosphorus limitation appears to be the only nutritional factor that results in a composition with polar lipids represented only by ornithine amide lipids (Taylor et al., 1998).

Interestingly, the 3-hydroxylated fatty acid of 10 carbon atoms is a component of the lipids of *B. gladioli* but not of those of *B. cepacia*, as indicated by lipid analysis performed on *B. gladioli* strains isolated from respiratory tract infections in cystic fibrosis patients (Christenson et al., 1989).

Differentiation of the plant pathogenic species of *Burkholderia* can be done by a direct colony thin-layer chromatographic

TABLE BXII.β.1. Fatty acid and ubiquinone composition of the genus *Burkholderia* (rRNA group II) and of aerobic pseudomonads of other rRNA groups[a,b]

	Ribosomal RNA groups				
Fatty acids	I	II	III	IV	V
3-OH					
$C_{10:0}$	+		+		+
$C_{11:0}$					+
$C_{11:0\ iso}$					+
$C_{12:0}$	+			+	+
$C_{12:0\ iso}$					+
$C_{13:0\ iso}$					+
$C_{14:0}$		+		+	
$C_{16:1}$		+			
2-OH					
$C_{12:0}$	(+)				
$C_{16:0}$		(+)			
$C_{16:1}$		(+)			
$C_{18:1}$		+			
Ubiquinones	Q-9	Q-8	Q-8	Q-10	Q-8

[a]Symbols: +, present; (+), not present in all strains of the group. A blank space means that the compound is not present in any strain of the group.

[b]Data taken from Oyaizu and Komagata (1983) and Stead (1992).

Editorial Note: Since submission of this manuscript, *Burkholderia norimbergensis* was reclassified as *Pandoraea norimbergensis* by Coenye et al. (2000). Readers are advised to review the chapter in that genus for additional details.

method. Only minor uncertainties have been noticed with respect to the composition of aminolipids (Matsuyama, 1995).

The general qualitative profile of hydroxylated fatty acids indicated above is constant for a given species, although at least in one case (*B. glumae*) a subdivision of strains into two types is possible based on differences in composition. One of the subgroups, which included the type strain, had a composition that was similar to the bulk of rRNA similarity group II. The other was represented by strains that had significant amounts of the $C_{10:0\ 3OH}$ fatty acid, and was unique in rRNA similarity group II in having the $C_{12:0\ 3OH}$ fatty acid (Stead, 1992). For some of the components of the fatty acid profile, significant quantitative variations can be observed among the strains of different species of *Burkholderia*.

In contrast to the hydroxylated fatty acids, which have their origin in lipid A, the more abundant, nonhydroxylated fatty acids are mainly located in the cytoplasmic membrane. Their value as taxonomic markers is significant at the species level and less at the higher level of the RNA similarity groups. All strains of group II have $C_{16:0}$, $C_{16:1\ cis}$, and $C_{18:1\ cis}$ nonhydroxylated fatty acids (Stead, 1992). The investigations of Komagata and his collaborators have established that Q-8 is the quinone characteristic of group II (Oyaizu and Komagata, 1983).

Hopanes have been detected in the composition of cells of *B. cepacia* (Rohmer et al., 1979), but the value of these compounds as chemotaxonomic markers is not known because later studies apparently did not include strains of other species of the group. This point perhaps warrants further attention.

Flagella Motility can be observed in young cultures of strains of all species of *Burkholderia*, with the exception of *B. mallei*, which lacks flagella. Cells of the latter species do not even show twitching motility on the surface of solid media (Henrichsen, 1975a). Motility in liquid is due to one or, more commonly, several polar flagella. A single flagellum per cell has been reported for *B. andropogonis*, *B. glathei*, and *B. norimbergensis* (see descriptions in the list of species at the end of this chapter). The best-known example is that of *B. andropogonis*, whose single flagellum is sheathed (Fuerst and Hayward, 1969a) (Fig. BXII.β.1).

SDS-polyacrylamide gel electrophoresis has been used for the characterization of the flagellins of different species of aerobic pseudomonads. Based on flagellin composition, *B. cepacia* strains have been divided into two groups. Group I has flagellin of molecular weight 31,000, whereas group II flagellin ranges from 44,000–46,000. Type I was serologically uniform, while group II was heterologous. The flagellin types of *B. cepacia* appear to be analogous to the two major flagellin types of *P. aeruginosa*, and they could be used as molecular epidemiological tools (Montie and Stover, 1983).

The methodology for the isolation of *B. pseudomallei* flagellin and its characterization has been described. Electrophoretic analysis under denaturing conditions results in monomer protein bands with an estimated M_r of 43,000 (Brett et al., 1994). O-polysaccharide-flagellin conjugates in this species have been described with respect to their structural and immunological characteristics (Brett and Woods, 1996).

Pili (fimbriae) Peritrichous pili have been identified years ago in *B. cepacia* (Fuerst and Hayward, 1969b). They are thought to facilitate adherence to mucosal epithelial surfaces (Kuehn et al., 1992; Sajjan and Forstner, 1993). Twitching motility is correlated with the presence of polar fimbriae, a correlation that is supported by the fact that this type of motility is absent from *B.*

cepacia, which has no polar fimbriae (Henrichsen, 1975a). No fimbriae have been observed in *B. andropogonis* (Fuerst and Hayward, 1969b).

One or more of five morphologically distinct classes of pili can be present in the cells of *B. cepacia*. Some of the types have been identified in cells of epidemically transmitted strains, and others in environmental isolates (Goldstein et al., 1995; Sajjan et al., 1995). The role of a 22-kDa pilin in binding *B. cepacia* to the mouth epithelial cells has been described (Sajjan and Forstner, 1993). Further details on fimbriae will be given in the section on pathogenesis for humans and animals.

Composition of cell envelope The earliest data on the cell wall composition of *B. cepacia* were obtained in S. Wilkinson's laboratory, where it was found that the major components, myristic, 3-hydroxymyristic, and 3-hydroxypalmitic acids, were indications that members of this group had a different composition than other species of aerobic pseudomonads. These results are in agreement with those of fatty composition of whole cells (Samuels et al., 1973). The core polysaccharide contains glucose, rhamnose, and heptoses, but at most a very low phosphorus content. The lipopolysaccharide (LPS) has a low content of 3-deoxy-D-manno-2-octulosonic acid (KDO), and the side chain is basically a mannan. One added peculiar feature is the presence of an acid-labile amino sugar phosphate presumably associated with the lipid A.

The results have been confirmed, at least in part, by Manniello et al. (1979). Analyses of *B. cepacia* performed by these workers revealed the presence of rhamnose, glucose, heptose, and hexosamine, but no KDO, and the phosphorus content was found to be about one-third of that of *P. aeruginosa*. The presence of KDO in the LPS of *B. cepacia*, however, was later confirmed (Straus et al., 1990).

A method of extraction of LPS from *B. pseudomallei* has been described. As in the case with *B. cepacia*, the link between the inner core and lipid A is stable to acid hydrolysis (Kawahara et al., 1992). The LPS of clinical strains of this last species was composed of two polymers made of different repeating units, but both polymers contained D-rhamnose and D-galactose residues (Cerantola and Montrozier, 1997).

Five major outer membrane proteins have been isolated from several strains of *B. pseudomallei*, with M_r values ranging from 17,000–70,000. One of the proteins associated with the peptidoglycan acts as a porin through which small saccharides may diffuse (Gotoh et al., 1994b).

Outer membrane proteins of *B. cepacia* and *B. pseudomallei* that are inducible by phosphate starvation appear to be similar to the *E. coli* PhoE porin protein. The latter does not have the binding sites for anions and phosphate that are present in the analogous proteins from *Pseudomonas* species (Poole and Hancock, 1986).

One porin of *B. cepacia* is an oligomer composed of two proteins. The purified 81-kDa whole protein (OpcPO) upon heating gives a major 36-kDa protein (OpcP1) and a minor one (OpcP2) of 27 kDa. The association of these two components is noncovalent (Gotoh et al., 1994a). Recently, the major porin protein, (OpcP1), was partially sequenced, and the information was used for cloning the gene. Its sequence showed an open reading frame of a length in agreement with that of OpcP1 (Tsujimoto et al., 1997).

Additional information about porins and other components of the cell envelopes may be found in the sections on antibiotic susceptibility and antigenic structure.

FIGURE BXII.β.1. *A, B. andropogonis* (syn. *"Pseudomonas stizolobii"*), with single-sheathed polar flagellum. Negative staining, 1% uranyl acetate, 0.4% sucrose. Bar = 1 μm. *B,* sheathed flagella of *B. andropogonis.* Same treatment as in *A.* Bar = 0.2 μm. (Courtesy of Dr. J.A. Fuerst.)

Pigmentation Pigmentation is by no means a universal character of *Burkholderia.* Some *B. cepacia* strains are not pigmented, whereas others produce phenazine pigments of a bewildering variety of colors when grown on solid chemically defined media containing different carbon sources. Pigmented strains of the species can be subdivided into two types on the basis of their pigmentation: some are yellow on glucose yeast extract peptone agar and others are various shades of brown, red, violet, and purple (Morris and Roberts, 1959). Morris and Roberts isolated the pigment from a purple-pigmented strain and demonstrated that its basic structure was that of a phenazine. In fact, two phenazine pigments—one yellow and the other purple—may be synthesized, both of which are water-soluble under neutral or alkaline conditions. A single strain can produce both types, only one type, or none. Because the pigments are soluble in water, both the colonies and the medium appear pigmented. Growth on King A medium[1] often enhances pigment production.

In the author's experience, pigment production in *Burkholderia* is, as in many other cases, a striking but not very reliable taxonomic character, because pigment biosynthesis often requires conditions that cannot be precisely controlled.

Nutrition and growth conditions The strains of *Burkholderia* species grow in media of minimal composition, without the addition of organic growth factors. Occasionally, strains are isolated from nature that grow extremely slowly but are stimulated by addition of complex organic mixtures such as yeast extract. Some of the fluorescent plant pathogens of the genus *Pseudomonas* fall in this category, and the phenomenon has also been observed for some *B. caryophylli* strains (Palleroni, 1984).

The ability to grow in media of very simple and chemically defined composition stimulated research on the nutritional versatility of the aerobic pseudomonads—among them, species later to be assigned to the genus *Burkholderia.* These studies have revealed a remarkable variety of organic compounds that can serve individually as carbon and energy sources for strains of some species (*B. cepacia, B. pseudomallei*). The types of compounds used for growth are the basis of the vast phenotypic information now available for many strains (Redfearn et al., 1966; Stanier et al., 1966; Ballard et al., 1970). One of the simplest chemically defined media used for nutritional studies is the one recommended for the hydrogen pseudomonads (Palleroni and Doudoroff, 1972).[2]

The nutritional investigations revealed that some strains of *B. cepacia* could utilize any of a list of 100 organic compounds (two-thirds of the list of tested substrates) (Stanier et al., 1966). Later work performed in various laboratories has enlarged the list considerably. This remarkable metabolic versatility of the species was unknown to plant pathologists, and its discovery rapidly converted *B. cepacia* into a fascinating subject for biochemical research. A sample of the nutritional properties of *Burkholderia* species is presented in Table BXII.β.2, and some of these properties, together with general characteristics of taxonomic importance, are summarized in Table BXII.β.3. Comparisons of nutritional properties of some related species are also to be found in later sections (Tables BXII.β.5 and BXII.β.7). To the remarkable metabolic versatility of *B. cepacia* we have to add the ability to fix N_2 that is exhibited by some of the strains (Bevivino et al., 1994;

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1. Medium of King et al. (1954) (g/l distilled water): Bacto-peptone (Difco), 20.0; Bacto Agar (Difco), 15.0; glycerol, 10.0; K_2SO_4, 10.0; $MgCl_2$, 1.4; pH 7.2.

2. Medium of Palleroni and Doudoroff (1972) (g/l 0.33 M Na-K phosphate buffer, pH 6.8): NH_4Cl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; ferric ammonium citrate, 0.05; $CaCl_2$, 0.005. The first two ingredients are added to the buffer and sterilized by autoclaving. The ferric ammonium citrate and $CaCl_2$ are added aseptically from a single stock solution that has been sterilized by filtration.

TABLE BXII.β.2. Utilization of carbon compounds by some *Burkholderia* species[a,b]

Compound[c]	B. cepacia	B. andropogonis	B. caryophylli	B. cocovenenans	B. gladioli	B. glathei	B. glumae	B. graminis	B. mallei	B. phenazinium	B. plantarii	B. pseudomallei	B. pyrrocinia	B. vandii	B. vietnamiensis
Carbohydrates/glycosides:															
N-Acetylglucosamine	+	−	+		+	+	+	+	+	+	+	+	+		+
Amygdalin	+	−	−		−	−	d⁻	−		−	+		+		d⁺
D-Arabinose	+	+	+		+	+	+	+	+	+	+	+	+		+
L-Arabinose	+	+	+	+	+	+	+	+	d	+	+	−	+		+
Arbutin	+	−	−		−	−	d⁻	−		−	+		−		d⁺
Cellobiose	d⁺	−	d⁻	+	d⁺	−	d⁺	d	+	−	+	+	+		+
L-Fucose	+	−	+	+	+	+	+	+		+	+	+	+		+
D-Fucose	+	d⁻	+	+	+	+	d⁺	d	+	−	+	+	−		+
Gentiobiose	d⁺	−	d⁻	−	−	−	d⁻	d		−	+		+		+
Glucosamine	+	−	+		+		+				+				+
2-Ketogluconate	+	d⁻	+		+	+	+	+	d	+	+	+	+		+
5-Ketogluconate	+	−	+		+	+	d	+		+	+		+		−
Lactose	−	+	−		−	+	−	+	−	−	−	−	−	+	d⁻
D-Lyxose	+	+	+	−	+	+	+	+		−	+		+		+
Maltose	d⁻	−	−	−	−	−	−	−	d	−	−	+	−		−
Melibiose	d⁻	−	−	+	−	−	+	−	−	−	−		−		−
Raffinose	d⁺	−	+	−	−	−	d⁺	+		−	−		−		d⁺
L-Rhamnose	d	d⁺	+	−	−	+	−	+	−	+	±	−	−	−	−
D-Ribose	+	+	+	+	+	−	+	d	−	+	+	+	+		d⁻
Salicin	+	−	−	+	−	−	d⁻	−	d	−	+	+	−		d⁺
Sucrose	d⁺	−	+	−	−	+	−	+	+	+	±	+	+	−	+
Tagatose	+	−	−		+	+	d⁺	−		−	−		+		+
Trehalose	+	d⁻	+	+	+	−	+	+	+	−	−	+	+	+	+
D-Xylose	d⁺	−	+	+	+	+	+	+	+	+	+	−	+	+	+
Polyalcohols:															
Adonitol	+	+	+	d⁻	+	+	+	+	−	+	−	d	+	d⁺	−
D-Arabitol	+	+	+		+	+	+	+		+	+		+		+
L-Arabitol	+	−	+		−	−	−	+		+	+	−	+		−
Dulcitol	+	−	−		+	+	+	−		−	+		+		+
Erythritol	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Xylitol	+	−	+	+	d⁺	+	−	+		+	−		+		−
Acids (anions):															
Aconitate	+	−	+	+	+		+		−		d⁺	+			+
Adipate	+	−	−	+	+	+	−		+		−	+			+
Azelate	+	−	±	+	+	+	+		d		±	d	d⁺		+
Butyrate	+	−	+	+	+	−	+		d		+	+			+
Caprate	+	−	−		+		+		−		±	+	+		+
Caproate	+	−	−		+		−		−		±	+	d⁺		+
Caprylate	+	−	−	+	+		d⁻		d		±	+	+		+
Citraconate	+	−	−	+	d⁻		−		−		±	−	d⁺		−
Citrate	+	+	+	+	+	+	+		d		±	+		±	+
Glutarate	+	−	−	−	d⁺		−		d		−	d	−		+
Glycolate	+	−	d⁻	−	d⁻	+	−		−		−	−	−	−	−
Heptanoate	+	−	−		+		+		−		+	+	d⁺		+
Isobutyrate	+	−	+	−	+	−	+		−		+	+			+
Isovalerate	d⁺	−	d⁻	−	−	−	−		−		−				+
Itaconate	−	−	−	−	−	+	−		−		−	−		−	+
α-Ketoglutarate	+	+	+	+	+		+		+		−	+			d⁺
Levulinate	+	−	−	−	+		−		−		−	+		d	+
Malonate	+	−	−	+	+	+	d⁻		d		d⁺	−			d⁻
Mesaconate	−	−	−	d⁺	+		−		−		±	−		+	−
Oxalate	−	−	+	−	−		d⁻		−		−	−			−
Pelargonate	+	−	−	+	+	−	d⁺		−		+	+			+
Pimelate	+	−	−	−	d⁺		−		d		−	d		d	−
Propionate	+	−	+		+		+		+		+	+	+		+
Sebacate	+	−	+	+	+	+	+		−		+	+	+	+	+
Suberate	+	−	d⁺		d⁺		d⁺		d		−	+		±	d⁻
Valerate	+	−	d⁻		+	−	−		−		−	+			+
D(−)-Tartrate	−	−	−	+	+	+	−		−		−	−		±	−
L(+)-Tartrate	d⁺	−	−	+	+	+	−		−		±	−		±	−
m-Tartrate	d⁺	−	+	+	+	+	−		−		−	−			d⁻

TABLE BXII.β.2. *(cont.)*

Compound[c]	B. cepacia	B. andropogonis	B. caryophylli	B. cocovenenans	B. gladioli	B. glathei	B. glumae	B. graminis	B. mallei	B. phenazinium	B. plantarii	B. pseudomallei	B. pyrrocinia	B. vandii	B. vietnamiensis
Amino acids and related compounds:															
β-Alanine	+	−	+	+	+	+	d+		+		−	+			+
DL-2-Aminobutyrate	+	−	−		+		−		d		d+	−		d+	d+
DL-3-Aminobutyrate	+		+		+		−				−				d+
DL-Aminovalerate	+	−	−		+		−				−				+
L-Arginine	+	−	+	+	+	+	+		+		d−	+			+
L-Citrulline	+	−	−	d+	+		−		−		−	−			d+
L-Cysteine	+	+	+		+	+	+				+				+
Glycine	d−	−	−	−	−	−	−		+		−	−		−	−
L-Histidine	+	−	+	+	+	+	+		+		+	+			+
L-Isoleucine	+	−	+	−	+	−	+		−		d−	+		+	+
DL-Kynurenine	+	d−	d−		+		−		−		−	+			+
L-Leucine	+	−	+	−	+	+	d+		−		+	−		+	+
L-Lysine	+	−	d−	+	+	+	−		−		−	+			+
L-Norleucine	+	−	d−	−	+		−		−		±			d−	+
DL-Norvaline	+	−	−		+		−				d+				d−
L-Ornithine	+	−	+	−	+	+	−		−		−	+		+	+
L-Phenylalanine	+	−	+	d−	+	+	+		d		+	+			+
L-Threonine	+	−	+	+	+	+	+		+	+	−	+		+	+
L-Tryptophan	+	−	+	−	+	+	+		+		−	+			+
L-Tyrosine	+	−	+	+	+		+		+		+	+			+
L-Valine	+	−	+	d−	+	+	+		d		±	+		+	+
Amines:															
α-Amylamine	+		+		−		−		−		−	+		−	d+
Benzylamine	d+	−	d+	−	d−		−		−		−	−		d−	+
Betaine	+	−	+		+	+	+		+		+	+			+
Butylamine	+	−	−		−		−				−				−
Diaminobutane	+	−	−		−		−				−				+
Ethanolamine	+	d+	−	+	+		+		−		+	+		−	+
Histamine	d+	−	−	−	−		−		−		−	−			+
Sarcosine	+	−	+	+	+	+	d+		d		−	d		+	+
Spermine	d−	−	−		−	+	−				−				d−
Tryptamine	+	−	−	−	−		+		−		−			−	−
Aromatic compounds:															
2-Aminobenzoate	+	d−	−		+		+		−		−	−			+
4-Aminobenzoate	−	−	−	+	−		−		−		−	−	+	+	−
Benzoate	d+	−	−	d−	+	+	−		+		−	+	−	d+	+
m-Hydroxybenzoate	+	−	−	d−	d+	−	−		−		−	−	−	−	−
o-Hydroxybenzoate	d+	−	−		−		+		−		−	−	−		−
Phenylacetate	+	−	−		+		−		+		−	+			+
Terephthalate	−	−	−		+		−		−		−	−			−

[a]For symbols see standard definitions; ±, slow. The + or − superscripts of the d symbol refer to the result obtained with the type strain, when known.

[b]Data from Gillis et al. (1995), Viallard et al. (1998), and Palleroni (1984).

[c]Carbon compounds used by all strains (with few exceptions; see Gillis et al., 1995) are fructose, fumarate, galactose, gluconate, glucose, mannose, glycerol, *m*-inositol, mannitol, sorbitol, acetate, DL-glycerate, DL-lactate, L-malate, pyruvate, succinate, D-α-alanine, L-α-alanine, DL-aminobutyrate, L-aspartate, L-glutamate, L-serine, L-proline, *p*-hydroxybenzoate. Carbon compounds not used by any strain: glycogen, methyl-mannoside, D-melezitose, inulin, starch, D-turanose, aesculin, creatine, 3-aminobenzoate, D-mandelate, phthalate, isophthalate.

Tabacchioni et al., 1995). This ability is also present in a closely related taxon, *B. vietnamiensis* (Gillis et al., 1995).

Temperature for growth Usually a temperature of 30°C is used for growth of all strains of the genus. Many of them, however, can grow well at 37°C and even at 40°C. Additional information on temperature relationships will be given in the section dealing with the description of individual species.

Oxygen relationships All strains grow well under aerobic conditions. Some species (*B. mallei, B. pseudomallei, B. caryophylli, B. plantarii, B. vandii*) can also use nitrate as the terminal electron acceptor under anaerobic conditions. The original description of *B. vietnamiensis* (Gillis et al., 1995) indicates that the strains are able to reduce nitrate to nitrite, suggesting that this is the final stage of the reduction process. However, *B. vietnamiensis* is described elsewhere as a denitrifier. In view of this, the species was omitted from Table 4, which presents the general properties of denitrifying aerobic pseudomonads. In spite of this, denitrification was included among the general properties useful as differential characteristics for *Burkholderia* species.

Metabolism and metabolic pathways Knowledge of many areas of the general metabolism of species of the genus *Burkholderia* is scanty and fragmentary. The following refers to some aspects of the general metabolism of the genus, with the exclusion of the catabolism of aromatic compounds, which will be treated in a second part.

Extensive nutritional information is available on a large num-

TABLE BXII.β.3. Characteristics useful for the differentiation of some *Burkholderia* species [a]

Characteristic	B. cepacia	B. andropogonis	B. caryophylli	B. cocovenenans	B. gladioli	B. glathei	B. glumae	B. mallei	B. plantarii	B. pseudomallei	B. vandii	B. vietnamiensis
Flagellar number	>1	1	>1	>1	>1	1	>1	0	>1	>1	>1	—
Diffusible pigments [b]	+	—	+	+	+	—	+	—	+	—	—	—
Arginine dihydrolase	—	—	+	—	—	—	—	+	—	+	+	—
Denitrification	+	—	+	+	+	+	+	+	+	+	+	+
Growth at 40°C	d	—	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	—	w	—	—	—	—	—	d	—	+	—	—
Starch hydrolysis	—	—	—	—	—	—	d	—	+	—	—	—
Extracellular PHB hydrolysis	—	—	—	—	d	—	d	—	+	—	—	—
Oxidase reaction	d	—	—	—	d	+	d	+	+	+	+	+
Growth on:												
Adonitol	+	+	+	d⁻	+	d	+	—	—	d	d⁺	—
α-Amylamine	+	—	—	—	—	—	—	—	—	+	—	d⁺
Citraconate	+	—	—	d⁺	—	—	+	—	w	—	d⁺	—
Erythritol	—	—	—	—	—	—	—	—	—	+	—	—
m-Hydroxybenzoate	+	—	—	d⁻	d⁻	—	—	—	—	—	d⁻	—
Levulinate	+	—	—	—	+	—	—	—	—	+	+	+
Mesaconate	—	—	—	d⁺	—	+	—	—	w	—	+	—
L-Rhamnose	d	d⁺	+	—	+	+	—	—	w	—	—	—
D-Ribose	+	+	+	+	+	+	+	—	+	+	—	d⁻
D(−)-Tartrate	—	—	—	+	+	+	—	—	—	—	w	—
meso-Tartrate	d⁺	—	+	+	—	—	+	—	—	—	—	d⁻
Tryptamine	+	—	—	—	—	+	+	—	—	—	—	—
D-Xylose	d⁺	+	+	+	+	+	+	—	—	—	+	+

[a]For the symbols and abbreviations see Table BXII.β.1. Data for *B. graminis*, *B. multivorans*, *B. norimbergensis*, *B. phenazinium*, *B. pyrrocinia*, and *B. thailandensis* have not been included since, aside from the general phenotypic features, for most of the characters in the table there is no information in the original sources.

[b]Strains of *B. cepacia* may produce nonfluorescent pigments of various colors; strains of *B. gladioli* and of *B. caryophylli* may excrete yellow-green nonfluorescent pigments; strains of *B. cocovenenans* produce greenish-yellow diffusible pigments.

TABLE BXII.β.4. Characteristics useful for differentiation of denitrifying aerobic pseudomonads[a]

Characteristics	Burkholderia caryophylli	B. mallei	B. plantarii	B. plantarii	B. vandii	Hydrogenophaga pseudoflava	Pseudomonas aeruginosa	P. alcaligenes	P. balearica	P. fluorescens and P. chlororaphis	P. mendocina	P. pseudoalcaligenes	P. stutzeri	Ralstonia solanacearum	R. pickettii
RNA group	II	II	II	II	II	III	I	I	I	I	I	I	I	II	II
Mol% G + C of DNA	65.3	69	64.8	69.5	68.5	66.5–68	67.2	64–68	64.1–64.4	59.4	62.8–64.3	62–64	60.7–66.3	66.5–68	64
Number of flagella	>1	0	>1	>1	>1	1	1	1	1	>1	1	1	1	>1	1
PHB accumulation	+	+	+	+	+	+	−	−	−	−	−	d	−	+	+
H$_2$ autotrophy	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth at 40°C	+	+	−	+	−	+	+	+	+	−	+	+	+	−	+
Gelatin liquefaction	−	+	+	+	+	−	+	d	−	+	−	d	−	−	−
Fluorescent pigment	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−
Pyocyanin production	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Yellow cellular pigment	−	−	−	d	−	+	−	d	−	−	+	−	−	−	−
Arginine dihydrolase	+	+	−	+			+	+	−	+	+	d	−	−	−
Starch hydrolysis	−	d	−	+	−	−	−	−	+	−	−	−	+	−	−
Extracellular PHB hydrolysis	−	d	+		−		−	−	−	−	−	−	−	−	−
Growth on:															
L-Arginine	+	+	d-	+			+	+	d	+	+	+	−		−
Azelate	−	d	w	d	d		+	−		d	+	−	−	d	+
Betaine	+	+	+	+			+	−	−	+	+	+	−	−	−
2,3-Butylene glycol	+	−		−		−	+	−		d	−	−	d	−	d
Ethylene glycol	−	−		−		−	−	−		−	+	d	+	−	−
Geraniol	−	−		−			+	−		−	+	−	−	−	−
Glycolate	+	−	−	−	−	d	−	−	−	−	+	−	+	d	+
L-Histidine	+	+	+	+		+	+	d	−	+	+	d	−	+	d
Levulinate	−	−	−	+	d		+	−		d	+	−	−	d	+
Maltose	−	d		+		+	−	−	+	−	−	−	+	−	−
Mannitol	+	+	+	+	+	+	+	−	−	+	−	−	d	d	−
Saccharate	+	−		−		d	−	−		d	+	−	d	+	+
Sarcosine	−	d	−	d	+	−	+	−		+	+	d	−	d	−
L-Serine	+	d	+	+			d	−	−	d	+	d	d	d	+
D-Xylose	+	+	+	−	+	+	−	−	+	d	−	−	−	−	+

[a]For symbols see standard definitions. The denitrifying pseudomonads *P. azotoformans*, *P. mucidolens*, and *P. nitroreducens* have not been included in this table.

ber of carbon substrates used individually as sole sources of carbon and energy for *B. cepacia*, *B. gladioli*, *B. caryophylli*, *B. pseudomallei*, and *B. mallei* (Redfearn et al., 1966; Stanier et al., 1966; Ballard et al., 1970; Palleroni and Holmes, 1981; Palleroni, 1984). In some of these reports, the information refers to names that are synonyms of some of the above (*"Pseudomonas marginata"* for *B. gladioli*, and *"Pseudomonas multivorans"* for some of the strains of *B. cepacia*). The information has been summarized in several tables in this chapter.

For *B. vietnamiensis* and *B. andropogonis*, a source of information on nutritional spectra useful for a comparison with other species of the genus, is given by Gillis et al. (1995). Less extensive surveys have been performed with *B. glumae*, *B. vandii*, and *B. cocovenenans* (Azegami et al., 1987; Urakami et al., 1994; Zhao et al., 1995).

As mentioned in the section on nutrition, some of the *Burkholderia* species are extremely versatile from the metabolic standpoint. This is particularly true of *B. cepacia*, the most versatile of all known aerobic pseudomonads, but *B. pseudomallei* and *B. gladioli* are similarly remarkable for their capacity of living at the expense of any of a long list of organic compounds as sole source of carbon and energy. Unfortunately, these species also have an infamous reputation as direct or opportunistic human and animal pathogens, and investigations on the saprophytic activities of some of the species are sparse because of the danger of handling the organisms in the laboratory.

All species of the genus *Burkholderia* that have been examined are able to accumulate PHB as a carbon reserve material, which they can degrade when nutrients in the medium become exhausted. However, use of exogenous PHB is an uncommon property among the aerobic pseudomonads. In *Burkholderia*, only *B. pseudomallei* and some strains of *B. mallei* are capable of degrading extracellular PHB (Redfearn et al., 1966; Stanier et al., 1966).

Table BXII.β.5 summarizes data on arginine utilization and the occurrence of arginine deiminase in some representative members of the various rRNA similarity groups of aerobic pseudomonads. Interestingly, *B. cepacia*—a member of RNA group II that is notorious for its nutritional versatility and for the diversity of its catabolic pathways—can degrade arginine only through the use of the succinyl transferase pathway, although it can use 2-ketoarginine and agmatine, the products of arginine oxidase and arginine decarboxylase, respectively (Stalon and Mercenier, 1984; Vander Wauven and Stalon, 1985).

In a survey of lysine catabolic pathways in the pseudomonads,

TABLE BXII.β.5. Arginine utilization and deiminase system in some aerobic pseudomonads

Organisms	RNA group	Arginine utilization	Arginine deiminase
Fluorescent saprophytic *Pseudomonas* species	I	+	+
Burkholderia cepacia, B. gladioli	II	+	−
B. mallei, B. pseudomallei	II	+	+
Ralstonia solanacearum, R. pickettii	II	−	−
Comamonas, Hydrogenophaga, Acidovorax	III	−	−
Brevundimonas	IV	−	−
Stenotrophomonas, Xanthomonas	V	−	−
Stenotrophomonas, Xanthomonas	V	−	−

it has been reported that *B. cepacia* and *P. aeruginosa* use the pipecolate pathway and not the so-called oxygenase pathway. *P. aeruginosa* can also use the cadaverine pathway, but this is not operative in *B. cepacia* (Fothergill and Guest, 1977; Palleroni, 1984). These facts are summarized in Table 1 (BXII.γ.108, p. 334) of the genus *Pseudomonas* in Volume 2, Part B.

N_2 fixation has been detected in strains of *B. cepacia* isolated from plant rhizospheres (Bevivino et al., 1994; Tabacchioni et al., 1995). A different set of nitrogen fixing strains studied in another laboratory was found to be closely related to this species, and was assigned the new species name *B. vietnamiensis* (Gillis et al., 1995).

Some miscellaneous activities of interest of the lesser-known species of the genus include the following. An α-terpineol dehydratase—capable of converting the citrus compound limonene to α-terpineol—was partially solubilized from a particulate fraction obtained from *B. gladioli* cells (Cadwallader et al., 1992). A lipase from *B. glumae* has been purified and some of its properties have been described (Deveer et al., 1991; Cleasby et al., 1992). Cloning and sequencing of a lipase gene of *B. cepacia* (*lipA*) have been performed, and its expression is dependent on a second gene (*limA*) (Jorgensen et al., 1991).

In cells of *B. caryophylli*, a D-threo-aldolase dehydrogenase that catalyzes the oxidation of L-fucose and the "unnatural" sugars L-glucose, L-xylose, and D-arabinose, has been purified and characterized. It is inhibited by D-glucose and other natural aldoses (Sasajima and Sinskey, 1979). Some enzymatic activities of species other than *B. cepacia* may be of environmental importance. Thus, *B. pseudomallei* is capable of breaking the C-P bond in the utilization of the herbicide *N*-(phosphonomethyl)-glycine, which is known by the empirical name glyphosate. The genes controlling this activity have been cloned and sequenced (Peñaloza-Vazquez et al., 1995). Similarly, the phytopathogen and human opportunistic pathogen *B. gladioli* was found to be able to form and cleave C-P bonds (Nakashita et al., 1991; Nakashita and Seto, 1991). In a more general way, it has been suggested that *B. gladioli* probably participates in the degradation of some xenobiotic compounds in the environment (Cadwallader et al., 1992).

Several siderophores are produced by species of *Burkholderia*. In iron-deficient media, *Pseudomonas aeruginosa, P. fluorescens*, and *B. cepacia* synthesize salicylic acid, a compound of particular interest because of its siderophore capacity (Visca et al., 1993) and the fact that it is a precursor of another siderophore, pyochelin. Interestingly, salicylate is used as a source of carbon and energy by many strains of *Burkholderia* and of many other aerobic pseudomonads. An iron-binding compound produced by the great majority of *B. cepacia* strains isolated from the respiratory tract

was named azurechelin and is capable of releasing Fe from transferrins (Sokol et al., 1992). Later, however, azurechelin was found to be salicylic acid (Visca et al., 1993).

Pyochelin production was found in half of 43 strains of *B. cepacia* isolated from cystic fibrosis patients. The siderophore has in its structure one molecule of salicylic acid and two molecules of cysteine (Cox et al., 1981). A siderophore of a linear hydroxamate/hydroxycarboxylate type, was discovered in *B. cepacia* and also found in *B. vietnamiensis*. It was named ornibactin and functions as a specific iron-transport system equivalent to that of the pyoverdin system of fluorescent pseudomonads. In the composition of ornibactins, there is a peptide, an amine, and acyl groups of different lengths (Stephan et al., 1993a, b).

In nature, these siderophores are often successful in competing for iron with siderophores of various other sources (Yang et al., 1993). Thus, pyochelin allows *B. cepacia* to grow in the presence of transferrin (Sokol, 1986).

In low Fe-content medium, *B. cepacia* excretes both pyochelin and a low molecular weight compound (1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone), which received the name of cepabactin. The structure resembles that of a cyclic hydroxamate, and it can also be considered a heterocyclic analogue of catechol (Meyer et al., 1989). The compound is related to synthetic hydroxy-pyridinones. Cepabactin had already been described as chelator (Winkler et al., 1986) and was known to have antibiotic properties (Itoh et al., 1979).

At least three different siderophores—ornibactins, pyochelin, and cepabactin—can be produced by a single *B. cepacia* strain. Other strains produce either two siderophores—ornibactins plus pyochelin or ornibactins plus cepabactin—and still other strains produce only pyochelin. In a survey of strains from a collection, 88% of the strains produced ornibactin, 50% pyochelin, and 14% cepabactin (J.M. Meyer, personal communication).

A recently identified member of the siderophore group produced by a strain of *B. cepacia* is cepaciachelin, a catecholate compound. The producing strain appears to be a unique example of a pseudomonad able to synthesize both hydroxamate and catecholate siderophores (Barelmann et al., 1996).

In summary, the variety of siderophores that *B. cepacia* is able to synthesize demonstrates once again the remarkable biochemical versatility of strains of this species.

All 84 strains of *B. pseudomallei* included in a study were found to produce a siderophore of approximately 1000 molecular weight. The compound, called malleobactin (Yang et al., 1991a), permitted cell growth in the presence of EDTA and of transferrin, and can trap iron from transferrin (at all pH values tested) and from lactoferrin. *B. cepacia* can use malleobactin as an iron-scavenging compound. However, pyochelin and azurechelin (salicylic acid) are more effective in trapping cell-derived iron as well as protein-bound iron (Yang et al., 1993).

Media with limiting phosphate concentrations enhance the production of a particular OM protein (OprP) of *Pseudomonas aeruginosa*. This protein is believed to be involved in phosphate transport and is only produced by species of *Pseudomonas*. Under similar conditions, both *B. cepacia* and *B. pseudomallei* produce proteins of a size similar to the PhoE protein of *Escherichia coli* and other enteric bacteria (Poole and Hancock, 1986).

Metabolism of aromatic and halogenated compounds For many years this subject has attracted the attention of biochemists because of the striking ability of prokaryotes to degrade aromatic compounds that are resistant to chemical attack and are not readily metabolized by other organisms. In recent years, this in-

terest has increased due to concern for chemically polluted environments and the obvious convenience of favoring these degradative activities as part of bioremediation strategies.

The first edition of this *Manual* included information on basic catabolic activities of the aerobic pseudomonads on simple aromatic compounds because of their obvious taxonomic implications (Palleroni, 1984). Much research has since been done on the catabolism of many aromatic compounds that include benzoate and derivatives, polycyclic aromatic hydrocarbons, biphenyl (particularly the halogenated derivatives), metabolic intermediates such as the halogenated catechols, and some important herbicides (24D and 245T). This field of research now contains a multitude of references, of which only a selected minority will be discussed here.

The investigations of Ornston and his collaborators on the biochemistry of degradation of aromatic compounds are of particular interest because the corresponding pathways were also analyzed for their phylogenetic implications. The β-ketoadipate pathway has taken center stage in these investigations. Natural selection has adopted many permutations in the distribution of its components, their regulation, and the genetic makeup, all of which have been highlighted in an excellent review (Harwood and Parales, 1996). Immunological cross-reaction was observed between the enzymes of one of the species (*Pseudomonas putida*) and the corresponding enzymes of other fluorescent *Pseudomonas* species. Cross-reaction, however, was also detected between the γ-carboxymuconolactone decarboxylases of *P. putida* and *B. cepacia* (Patel and Ornston, 1976). Although the results suggested that the interspecific transfer of the structural gene for the enzyme was not common among pseudomonads, it nevertheless seemed to have occurred between these two distantly related species. The cross-reaction did not extend to other enzymes of the two species, including the salicylate hydroxylases, which are structurally different, and for which an explanation based on convergent evolution has been proposed (Kim and Tu, 1989).

As mentioned in a description of the metabolism of *para*-hydroxybenzoate (POB), *B. cepacia* is able to convert *meta*-hydroxybenzoate (MOB) to gentisate by the action of a 6-hydroxylase (Yu et al., 1987). Interestingly, this finding relates to an earlier report on the formation of gentisate from MOB by *Comamonas acidovorans*, which is a member of the *Betaproteobacteria* in which *Burkholderia* is located (Wheelis et al., 1967). A description of the induction of the hydroxylase from *B. cepacia* has been published (Wang et al., 1987).

The genetic organization and sequence of genes of the α and β subunits of protocatechuate 3,4-dioxygenase of *B. cepacia* has been investigated, and there is extensive similarity to genes of other *Pseudomonas* species, although this similarity does not extend to the promoter sequences (Zylstra et al., 1989b). The pattern of induction of this and other enzymes of the POB metabolism has been examined (Zylstra et al., 1989a).

As a member of a microbial community, *B. cepacia* was the most competitive member among other aerobic pseudomonads in the degradation of toluene (Duetz et al., 1994). A novel pathway of toluene catabolism was described for this organism, with the participation of toluene monooxygenase, which is able to hydroxylate toluene, phenol, and cresol, and also to catalyze the degradation of trichloroethylene (TCE) (Shields et al., 1991). Cometabolism of TCE and toluene has been described (Landa et al., 1994). A constitutive strain was selected for the degradation of TCE (Shields and Reagin, 1992). Degradation of TCE by *B. cepacia* using the toluene monooxygenase system is expressed at

higher capacity than the toluene dioxygenase systems present in other organisms (for instance, *P. putida*) (Leahy et al., 1996).

The toluene 2-monooxygenase from *B. cepacia* is a three-component system capable of oxidizing toluene to *o*-cresol and this to 3-methylcatechol. The catabolic features of this system resemble those of soluble methane monooxygenase from methanotrophic bacteria (Newman and Wackett, 1995). For the activity of toluene 2-monooxygenase on TCE, all its protein components and NADH are required. All protein components were modified during TCE oxidation, but reducing compounds such as cysteine protected the enzyme (Newman and Wackett, 1997).

Phthalate oxygenase is an enzyme specific for phthalate and closely related compounds. The system of *B. cepacia*, which requires the contribution of phthalate oxygenase reductase for efficient catalytic activity, is similar to other bacterial oxygenase systems. The *B. cepacia* enzyme can be isolated in large quantities and its stability is higher than that from other sources (Batie et al., 1987).

B. cepacia strains grow on fluorene and degrade this compound by a mechanism analogous to naphthalene catabolism. The system has wide specificity, and the range of substrates includes many other polycyclic aromatic compounds (Grifoll et al., 1995). But in spite of the remarkable catabolic versatility of the species, there are some limitations to the range of susceptible substrates. Thus, strains isolated on phenanthrene from polyaromatic hydrocarbon (PAH)-contaminated soils had limited capacity to use higher PAHs (Mueller et al., 1997).

For more than a decade, the degradation of the halogenated herbicides 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (245T) by *B. cepacia* has been the object of attention by researchers, among them, A. Chakrabarty and his group (Haugland et al., 1990). In fact, *B. cepacia* grows luxuriantly on 245T. Although initially this species is not able to use phenoxyacetate, it can acquire this property by long selective pressure. Gene activation in the mutants seems to be due to translocation of insertion elements (Ghadi and Sangodkar, 1994). Polychlorinated phenols are produced in the degradation, and they are further metabolized by *B. cepacia* to the corresponding hydroquinones (Tomasi et al., 1995). The cloning, mapping, and expression of genes controlling the degradation by strains of this species have been under investigation (Sangodkar et al., 1988).

The study of spontaneous *B. cepacia* mutants unable to degrade 245T has permitted the identification of insertion sequences that facilitate or are required for growth at the expense of 245T (Haugland et al., 1991). The rapid evolution of the degradative pathway for this herbicide probably has been possible by insertion elements (such as IS1490) that play a central role in the transcription of 245T genes in *B. cepacia* (Hubner and Hendrickson, 1997). A 1477 bp sequence was repeated several times in a *B. cepacia* strain chromosome, and by the location it was concluded that genes involved in 245T degradation were actually recruited from foreign sources (Tomasek et al., 1989). Originally, foreign gene recruitment such as the mechanism postulated by Lessie and Gaffney (1986), may have been responsible for the acquisition of 245T degradation capacity. Better knowledge of the evolution of these pathways may facilitate the job of developing strains with enhanced degradative capacity (Daubaras et al., 1996a).

B. cepacia competes effectively in the microflora for the degradation of the herbicide 2,4-dichlorophenoxyacetic acid (24D) (Ka et al., 1994a). The enzyme that cleaves 3,5-dichlorocatechol in this pathway has been purified (Bhat et al., 1993). To monitor

a strain in the degradation of 24D, a reporter gene system containing *luxAB* and *lacZY* was integrated into the chromosome of the strain, which thus could be readily identified in the community (Masson et al., 1993).

Oxygenase TftAB, capable of converting 245T to 2,4,5-chlorophenol, is an enzyme of wide specificity, since it can give phenolic derivatives of several other related compounds (Danganan et al., 1995). Two proteins, TftA1 and TftA2, characterized in the pathway, were sequenced and found to have similarity to BenA and BenB of the benzoate 1,2-dioxygenase system of *Acinetobacter calcoaceticus*, and to XylX and XylY from the equivalent system in *Pseudomonas putida* (Danganan et al., 1994). The *B. cepacia* enzyme that degrades 2,4,5-trichlorophenol, the intermediate in 245T catabolism, has been characterized and found to consist of two components (Xun, 1996).

1,2,4-trihydroxybenzene, another intermediate in 245T degradation, is a substrate for the enzyme hydroxyquinol 1,2-dioxygenase. The enzyme is specific, and it is a dimeric protein of 68 kDa (Daubaras et al., 1996b).

Environmental strains of *B. cepacia* are active in the degradation of polychlorinated biphenyls. As in other examples of aerobic metabolism of haloaromatic compounds, dehalogenation often occurs after ring cleavage (Arensdorf and Focht, 1995). *In vitro* constructed hybrids of *B. cepacia* could carry out total degradation of 2-Cl, 3-Cl, 2,4-dichloro-, and 3,5-dichlorobiphenyl (Havel and Reineke, 1991).

Chlorocatechols are key intermediates in the metabolism of haloaromatic compounds, and they are metabolized through reactions resembling those for catechol. A 2-halobenzoate 1,2-dioxygenase, a two-component system of *B. cepacia* strain 2CBS has a very broad specificity (Fetzner et al., 1992). However, in *B. cepacia* isolated from enrichment on 2-chlorobenzoate and in mutants blocked in different steps of the pathway, the 2,3-dioxygenase of the *meta* pathway predominated over the 1,2-dioxygenase (or *ortho*) system (Fetzner et al., 1989).

The dioxygenases and the cycloisomerases involved in the modified *ortho* pathways resemble the enzymes of pathways for nonhalogenated compounds, while the diene-lactone hydrolases needed in a later step are quite different (Schlomann et al., 1993). The results of work done on systems isolated from *Alcaligenes* and *B. cepacia* suggest that the hydrolases of the modified pathway may have been recruited from a different preexisting pathway, which was already operating before the start of industrial synthesis of halogenated compounds (Schlomann, 1994). When plasmid TOL is introduced into *B. cepacia* strains, these strains are able to grow with 3,4- and 3,5-dichlorotoluene, thus bypassing dead-end routes of chlorocatechols degradation (Brinkmann and Reineke, 1992).

The results of the experiments that have been briefly discussed in the preceding paragraphs often point to the high similarity among some components of peripheral metabolic pathways among organisms that are rather distantly related, suggesting horizontal transfer of the corresponding genetic determinants. One additional case is the high similarity between the genes coding for the *cis*-biphenyl dihydrodiol dehydrogenases of *P. putida* (gene *bphB*) and that of *B. cepacia* (Khan et al., 1997), and also between the *bphC* genes of both species, which code for the 2,3-dihydroxybiphenyl 1,2-dioxygenase (Khan et al., 1996b). On the other hand, in spite of having almost identical amino acid sequences, the biphenyl dioxygenase of *B. cepacia* (gene *bphA1*) and that of *Pseudomonas pseudoalcaligenes* have markedly

different substrate ranges, the one for *B. cepacia* being wider (Kimura et al., 1997).

Additional details on the metabolism of aromatic compounds may be found in the section on plasmids.

Genetic characteristics The genome of a strain of *B. cepacia* (ATCC 17616) consists of three replicons whose respective sizes are 3.4, 2.5, and 0.9 Mb, which, together with the 170-kb cryptic plasmid present in the strain, gives an overall estimate for genome size of approximately 7 Mb (Cheng and Lessie, 1994). The three large replicons had ribosomal RNA genes, as well as the insertion elements previously described by Lessie and collaborators. Studies on mutants and the associated reductions in size of the replicons provide a convenient framework for genetic analysis of this strain.

The genome of another strain of *B. cepacia* (ATCC 25416) was analyzed and found to contain four circular replicons of sizes 3.65, 3.17, 1.07, and 0.2 Mb (Rodley et al., 1995). The values total 8.1 Mb. Again the interpretation is that the genome is made of three chromosomes and a large plasmid, because of the presence of ribosomal RNA genes only in the three large replicons. An interesting additional observation is the fact that multiple chromosomes are not confined to *B. cepacia* but also are found in other members of rRNA similarity group II (*B. glumae*, *Ralstonia pickettii*, and *R. solanacearum*) (Rodley et al., 1995). A very useful review that highlights the remarkable genomic complexity and plasticity of *B. cepacia* has been published (Lessie et al., 1996).

A *recA* gene has been identified in *B. cepacia* that is able to complement a *recA* mutation in *E. coli*, also restoring UV and methylmethane sulfonate resistance and proficiency in recombination (Nakazawa et al., 1990). Additionally, an SOS box related to LexA-regulated promoters and -10 and -35 consensus sequences have been detected in *B. cepacia*. The predicted RecA protein sequence shows 72% similarity with that of *P. aeruginosa*.

Transposable elements that activate gene expression in *B. cepacia* have been identified by Lessie and his collaborators (Scordilis et al., 1987). This opened a new horizon on insertional activation and on the significance of a high frequency of genomic rearrangements that presumably are related to the remarkable versatility of the species (Gaffney and Lessie, 1987; Lessie et al., 1996).

In spite of all these developments, at present there is incomplete knowledge of gene expression and of regulatory mechanisms in species of *Burkholderia* because of the lack of an appropriate gene exchange system. In the absence of a conventional bacterial genetic system, some alternatives have been developed. One of the proposed methods of genetic analysis is based on transposon mutagenesis and complementation of mutations by means of cloned genes. In a particular application, a shuttle plasmid was constructed that could be used for cloning genes of *B. cepacia* involved in protease production (Abe et al., 1996).

Studies on population genetics have been carried out on a population of *B. cepacia* isolated from a southwestern stream in the United States, to examine the allelic variation in a group of loci using multilocus enzyme electrophoresis. The studies showed a low degree of association between the loci, or extensive genetic mixing. This evidence of frequent recombination (and consequent low levels of linkage disequilibrium) indicates that the structure of the population was not clonal (Wise et al., 1995).

The topology of a 23S rRNA phylogenetic gene tree agrees with the 16S rRNA tree (Höpfl et al., 1989).

Plasmids A cryptic 170-kb plasmid that was discovered in *B. cepacia* ATCC 17616 has been the subject of much research. Derivatives of this strain carried versions of the plasmid containing various insertion sequences in different combinations. These elements, inserted in the broad host range plasmid RP1, served as probes to examine the extent of the reiteration of the various components in the genome of the organism. The results indicated a high frequency of genomic rearrangements, mainly the result of replicon fusions promoted by the insertion elements, that could help explain the remarkable biochemical versatility of the species (Barsomian and Lessie, 1986; Gaffney and Lessie, 1987).

Derivatives of a nonconjugative *Pseudomonas* plasmid (pVS1), carrying genes for mercury and sulfonamide resistance as well as segments required for stability and for mobilization by plasmid RP1, have been established in *B. cepacia* (Itoh et al., 1984). Some further constructions based on pVS1 could be used as cloning vectors (Itoh and Haas, 1985).

In many instances, the degradation of toxic compounds and environmental pollutants is controlled by genes located in plasmids. Some of them already have been mentioned, and only brief reference to some additional instances will be made here. A 50-kb plasmid is responsible for the degradation of *para*-nitrophenol by *B. cepacia*, following an oxidative route with the production of hydroquinone and nitrite. The plasmid can be conjugationally transferred (Prakash et al., 1996). A 70-kb plasmid in *B. cepacia* strain 2CBS carries a gene cluster with the determinant of an enzyme able to catalyze double hydroxylation of 2-halobenzoates with release of halogenide and CO_2 and producing catechol (Haak et al., 1995). A catabolic plasmid involved in the degradation of 4-methyl-*o*-phthalate was described and named MOP (Saint and Ribbons, 1990). Finally, a fragment of a plasmid involved in the catabolism of 4-methylphthalate in *B. cepacia* was sequenced and two open-reading frames were discovered, one of which encoded a permease that belongs to a group of symport proteins found in both pro- and eucaryotes. Information on this system could be used to improve the degradative capability for bioremediation purposes (Saint and Romas, 1996).

The novel pathway of toluene degradation mentioned in the section on metabolism of aromatic compounds is inducible and the corresponding genes are located in a plasmid. A strain of *B. cepacia* was found to carry two plasmids, one of 108 kb (named TOM) containing the genes for a toluene monooxygenase pathway that was expressed constitutively. The same strain also contained a small plasmid of less than 70 kb (Shields et al., 1995).

A new plasmid (pMAB1) with genes controlling the degradation of 24D in *B. cepacia* was characterized, and spontaneous negative mutants were isolated under nonselective conditions. Instead of the original 90-kb plasmid, these mutants had a smaller one (70-kb) or had lost it altogether. The activity could be regained by reintroducing the larger plasmid by electroporation. The 70-kb plasmid lacked a region that included the gene *tfdC* encoding the 3,5-dichlorocatechol 1,2-dioxygenase, whose sequence was identical with that of a well-characterized 24D degradative plasmid (pJP4) of *Alcaligenes eutrophus*. The similarity did not extend to the rest of the plasmids (Bhat et al., 1994). Another *B. cepacia* plasmid (pBS1502) was found to be able to control the early dehalogenation of 2,4-dichlorobenzoate (Zaitsev et al., 1991). There is also a report of the presence of a catabolic plasmid named MOP carrying genes involved in the catabolism of phthalate derivatives (Saint and Ribbons, 1990), and a small (2-

kb) plasmid was implicated in the degradation of phenylcarbamate herbicides (Gaubier et al., 1992).

Plasmid analyses in combination with other typing techniques have been proposed for epidemiological studies on nosocomial infections by *B. cepacia* (Yamagishi et al., 1993). Early studies based on agarose gel electrophoresis of *B. cepacia* extracts demonstrated the presence of one or more plasmids in several strains of *B. cepacia* of plant and clinical origin. The plasmid composition, together with bacteriocin production and sensitivity, and pectolytic activity, could have applications in epidemiological studies (González and Vidaver, 1979).

Strains of *B. cepacia* from clinical and pharmaceutical origin carried large plasmids (146–222 kb) containing antibiotic resistance genes (Lennon and DeCicco, 1991). The nonconjugative *B. cepacia* plasmid pVS1 contained Hg and sulfonamide resistance genes, and a segment required for mobilization by RP1 (Itoh et al., 1984).

Bacteriophages Most cultures of *B. pseudomallei* from collections have shown spontaneous phage production (Denisov and Kapliev, 1991). From the strains from a collection, 14 pure lines of bacteriophages belonging to two morphological types were isolated. The specificity of these phages was studied, and it was found that some strains of the host undergo poly-lysogeny, which was inferred from the fact that phages of different morphological types could be isolated from single strains (Denisov and Kapliev, 1995).

A generalized transducing phage was isolated from a lysogenic strain of *B. cepacia*, and half of more than 100 strains of the species were sensitive to it (Matsumoto et al., 1986).

Strain Berkeley 249 (ATCC 17616) of *B. cepacia*, which has been studied very intensively in Lessie's laboratory, carries an organic solvent-sensitive phage (Cihlar et al., 1978). Its sensitivity is attributed to alteration of a tail component provoked by the solvent. Results obtained by using other *B. cepacia* strains as hosts imply the occurrence of host restriction and modification systems. The phage has a head of 55 nm in diameter, a broad contractile tail of 15×145 nm, and double-stranded DNA of a molecular weight of about 3×10^7.

Many years ago a bacteriophage lytic for a wide range of aerobic pseudomonads was isolated and tested against strains of different species, among which was *"Pseudomonas multivorans"* (later identified as a synonym of *B. cepacia*) (Kelln and Warren, 1971). *B. cepacia* was insensitive; the phage was lytic only for species of rRNA similarity group I (*Pseudomonas sensu stricto*) and not for members of other rRNA groups.

Bacteriocins Early work performed on *B. cepacia* strains isolated from plants and from clinical specimens showed that the two groups could be differentiated by bacteriocin production patterns, onion maceration tests, and hydrolysis of pectate at low pH, thus suggesting the usefulness of these characteristics in epidemiological studies (González and Vidaver, 1979). Additional differences between strains from the two sources have been recorded (Bevivino et al., 1994) and will be discussed below (see Ecology, Habitats, and Niches).

A number of *B. cepacia* bacteriocins ("cepaciacins") were defined in work done on a collection of 34 strains isolated from plant rhizospheres and human patients (Dodatko et al., 1989a). One of the cepaciacins consisted of protein and carbohydrate in a 3:1 molecular ratio. The bacteriocin was thermolabile, stable within a narrow range of pH values, and it was destroyed by

proteolytic action. UV irradiation or mitomycin C stimulated its biosynthesis (Dodatko et al., 1989b).

A typing scheme has been described based on bacteriocin susceptibility and production by *B. cepacia* strains using six producer strains and a set of eight indicator strains (Govan and Harris, 1985). The majority of strains of a large collection were typed into a total of 44 combinations, and the typing scheme was found to be useful for the possibility of its application to epidemiological studies.

Antigenic structure The LPS structure of different *B. pseudomallei* strains is quite homogeneous. Antibodies prepared with material from one strain react with all others (Pitt et al., 1992). In agreement with the degree of their phylogenetic relationships, cross-reactions are observed with *B. mallei* and, to a lesser extent, with *B. cepacia*.

Many features of the biological activity of LPS isolated from *B. pseudomallei* cells have been described, and the strong mitogenic activity that it has toward murine splenocytes has been attributed to unusual chemical structures in the inner core attached to lipid A (Matsuura et al., 1996). In addition, information is available about the identification, isolation, and purification of an exopolysaccharide of this species. As in the instance described above, the compound having a molecular mass of >150 kDa did not show cross-reactivity with any of the species of all the *Pseudomonas* rRNA similarity groups, with the exception of the closely related species *B. mallei* (Steinmetz et al., 1995).

Purification to homogeneity of flagellin from several *B. pseudomallei* strains gave monomer flagellin bands of M_r 43,400 Da. Passive immunization studies showed that a specific antiserum could protect animals from challenge by a *B. pseudomallei* strain of different origin (Brett et al., 1994).

Two monoclonal antibodies were found to be highly specific for *B. pseudomallei* when tested by indirect enzyme-linked immunosorbent assay and immunoblotting against whole-cell extracts of other *Burkholderia* species, fluorescent pseudomonads, and *E. coli*. One of the antibodies could agglutinate all 42 *B. pseudomallei* strains included in the study, thus providing a tool for rapid identification of the species using primary bacterial cultures from clinical specimens (Pongsunk et al., 1996).

Of the serological typing schemes devised for *B. cepacia*, the one most widely used is that proposed by Werneburg and Monteil (1989). Originally, the scheme described procedures for the preparation, adsorption, and titration of O and H rabbit sera, and it could define seven O (O1 to O7) and five H antigens (H1, H3, H5, H6, and H7) for the slide agglutination test and the agglutination and immobilization test, respectively (Heidt et al., 1983). The scheme later was supplemented with new serotypes, using strains of a different geographical origin, to make a total of 9 O and 7 H antigens (Werneburg and Monteil, 1989). Other immunological typing schemes have been proposed (Nakamura et al., 1986).

Wilkinson and his collaborators have studied the composition of the O-specific polymers from the LPS of *B. cepacia* strains belonging to groups O1 (Cox and Wilkinson, 1990b), O3, O5 (Cox and Wilkinson, 1989b), O7 (Cox and Wilkinson, 1990a), and O9 (Taylor et al., 1994a). The O9 group has repeating units that are also present in *Serratia marcescens*. In the same laboratory it has been discovered that the O antigen of the LPS of *B. cepacia* serotype E (O2) is composed of two different trisaccharide repeating units in a 2:1 ratio (Beynon et al., 1995), and that the same O specific polymer is found in the two related species *B. cepacia* and *B. vietnamiensis*.

Interestingly, the lipopolysaccharides extracted from *B. cepacia* and *B. gladioli* have a higher endotoxic activity and provoke a higher cytokine response than that from *Pseudomonas aeruginosa* (Shaw et al., 1995). This adds to the importance to the human pathogenic propensities of *P. gladioli*, an example of a plant pathogen of medical importance similar to that of *B. cepacia*. Plant-associated *B. gladioli* can be differentiated from other pathogenic and symbiotic bacterial species by a rapid slide agglutination test using polyclonal antisera conjugated to protein-rich *Staphylococcus aureus* whole cells (Lyons and Taylor, 1990).

Cross-reactivity of *P. aeruginosa* antipilin monoclonal antibodies with heterogeneous strains of *P. aeruginosa* and *B. cepacia* has been reported (Saiman et al., 1989).

Antibiotic susceptibility For obvious reasons, most information on antibiotic susceptibility of species of the genus *Burkholderia* refers to only a few species of medical importance (*B. pseudomallei, B. cepacia, B. gladioli*).

All strains of *B. cepacia* that have been tested are sensitive to sulfonamides and novobiocin. Most are also sensitive to trimethoprim plus sulfamethoxazole, and to minocycline and chloramphenicol (Santos Ferreira et al., 1985). Both *B. cepacia* and *B. gladioli* are resistant to a wide variety of antibiotics (ticarcillin by itself or mixed with clavulanic acid; cefsulodin, imipenem, aminoglycosides, colistin, and fosfomycin) (Baxter et al., 1997). A catechol-containing monobactam (BMS-180680) was quite active (MIC90, 1 μg/ml; MIC90 is a minimum concentration which inhibits 90% of the strains tested) (Fung-Tomc et al., 1997).

Eighty percent of a collection of strains of *B. cepacia* was tested for inhibition by ceftazidime (Tabe and Igari, 1994). A number of quinolone analogs and derivatives (trovafloxacin, ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, clinafloxacin, ceftazidime) were active on several Gram-negative, nonfermentative species, including *B. cepacia*. In comparison, the MIC for imipenem on *B. cepacia* and on *Stenotrophomonas maltophilia* was very high (Visalli et al., 1997).

In a comparison of the activity of many antibiotics against *B. pseudomallei*, a quinolone (tosufloxacine) and a tetracycline derivative (minocycline) appeared to be the most active (Yamamoto et al., 1990).

For years, the oral maintenance treatment of melioidosis has depended on the combined action of amoxicillin and the β-lactamase inhibitor clavulanic acid (Suputtamongkol et al., 1991). Biapenem, one of several carbapenem antibiotics tested against *B. pseudomallei*, was the most active against strains that showed a diminished susceptibility to third-generation cephalosporins (Smith et al., 1996).

Several resistance mechanisms operate in different species of *Burkholderia*. Resistance to cationic antibiotics in *B. cepacia* has been attributed to their ineffective binding to the outer membrane as a consequence of the low number of phosphate and carboxylate groups in the LPS, and the presence of protonated aminodeoxypentose (Cox and Wilkinson, 1991). The involvement of the outer membrane of *B. cepacia* in the resistance to polymyxin and aminoglycosides may also be related to a particular arrangement in the structure of the outer membrane, in which cation-binding sites on LPS are protected from polycations (Moore and Hancock, 1986).

An important factor in the antibiotic resistance in these organisms is porin permeability (Parr et al., 1987; Burns et al., 1996). However, a different interpretation is that resistance may not be a direct consequence of permeability of the porins, but

instead may be related to the low number of porins per cell. A β-lactam-resistant mutant of *B. cepacia* and resistant strains of this species isolated from cystic fibrosis cases owe their resistance to a low porin content. These strains had reduced amounts of a 36-kDa outer membrane protein and did not express a 27-kDa outer membrane protein that can be a major porin or a major component of the porin complex of the cells (Aronoff, 1988). According to this view, the most common resistance mechanism in *B. cepacia* is the low porin-mediated outer membrane permeability, combined with multiple drug resistance due to an efflux pump system (Burns et al., 1996).

It may be of interest to mention here that growth in the presence of salicylate or other weak acids can induce resistance to antibiotics in *B. cepacia*, because these compounds have been found to be inhibitors of porin formation (Burns and Clark, 1992).

A number of β-lactams are susceptible to hydrolysis by a β-lactamase of *B. cepacia*, a metalloenzyme of type I that is induced by imipenem (Baxter and Lambert, 1994). A significant degree of similarity was found between the chromosomal β-lactamase of *B. cepacia* strain 249 and the enzymes of *Pseudomonas aeruginosa* and *E. coli*. Interestingly, in spite of differences in the mol% G + C content of the DNA of these organisms, the codon usage in *B. cepacia* resembled that of *E. coli* (Proenca et al., 1993).

The multiple resistance gene *oprM* of *P. aeruginosa* is part of a highly conserved efflux system. A gene homologous to *oprM* was identified in *B. cepacia* (Burns et al., 1996). Moreover, an intragenic probe hybridized the genomic DNA of several fluorescent pseudomonads and, in addition, the DNA of *B. pseudomallei* (Bianco et al., 1997). A lucid review is available on the participation of multidrug efflux pumps in the resistance of Gram-negative organisms to antibiotics (Nikaido, 1996).

The fusaric acid resistance gene of *B. cepacia* has been cloned and sequenced (Utsumi et al., 1991).

Antibiotic production Antifungal antibiotics have been identified in strains of *B. cepacia*. Cepacidine A, composed of two related forms, cepacidine A1 and A2, is a cyclic peptide and xylose connected to a 5,7-dihydroxy-3,9-diaminooctadecanoic acid (Lee et al., 1994a; Lim et al., 1994). Two antibiotics previously discovered and described under the names cepacin A and B are not related to the cepacidines. The cepacins showed good antistaphylococcal activities (MICs of 0.2 and 0.05 μg/ml for cepacin A and B, respectively), but no significant activity against Gram-negative bacteria (Parker et al., 1984).

Another antifungal antibiotic is pyrrolnitrin (Jayaswal et al., 1993). The producing organism was named *Pseudomonas pyrrocinia* (Imanaka et al., 1965), now *Burkholderia pyrrocinia*. The antibiotic is also produced by *B. cepacia* and by some strains of *Pseudomonas chlororaphis* (Elander et al., 1968). A study by Burkhead et al. (1994) has examined the conditions of pyrrolnitrin by *B. cepacia* in culture and in the wounded areas of potatoes colonized by the organism.

Some compounds related to pyrrolnitrin (amino-pyrrolnitrin, and monochloroamino-pyrrolnitrine) also have antifungal properties (McLoughlin et al., 1992). The antibiotic activity of *B. cepacia* has been reported to antagonize the pathogenic activity of the sunflower wilt fungus (McLoughlin et al., 1992) and to be a suppressor of maize soil-borne disease (Hebbar et al., 1992). The influence of some environmental factors on the antagonism of *B. cepacia* toward *Trichoderma viride* has been analyzed (Upadhyay et al., 1991), as well as some morphological alterations and inhibition of conidiation of plant pathogenic fungi (Upadhyay

and Jayaswal, 1992). A compound having both hemolytic activity and antifungal action was characterized and given the name cepalycin (Abe and Nakazawa, 1994).

Transposon mutagenesis could eliminate pyrrolnitrin production ability. However, the mutation failed to be complemented by the cloned gene because of difficulties encountered in mobilizing the carrier cosmids from *E. coli* to *B. cepacia* mutants (Jayaswal et al., 1992).

A group of eight cyclic peptides of antifungal activity, the xylocandins, has been isolated from *B. cepacia* and characterized (Bisacchi et al., 1987). A mixture of two of the forms (A1 and A2) showed potent anticandidal and antidermatophytic activities *in vitro* (Meyers et al., 1987).

The antagonistic activity of *B. cepacia* is not limited to antifungal activity. Of practical importance is the finding that metabolites produced by strains of this species can antagonize plant pathogenic agents such as *Ralstonia solanacearum* (Aoki et al., 1991).

Toxoflavin, an azapteridine antibiotic produced by *B. cocovenenans*, was identified more than 30 years ago (Lauquin et al., 1976). The production of toxoflavin is inhibited by the combined action of 2% NaCl and acidity (pH 4.5) in the medium (Buckle and Kartadarma, 1990). A monobactam antibiotic (MM 42842) is also produced by *B. cocovenenans*. It is related to a previously described antibiotic named sulfazecin. In addition, the *B. cocovenenans* strain synthesizes bulgecin, an antibiotic also produced by other aerobic pseudomonads (Box et al., 1988; Gwynn et al., 1988).

Tropolone, which is known to have antibacterial and antifungal activities (Lindberg, 1981), is produced by *B. plantarii* cultures (Azegami et al., 1987).

Plant pathogenicity Many species of the genus *Burkholderia* are pathogenic for animals or plants. Phytopathogenic pseudomonads are located in three of the five RNA similarity groups. One of them is rRNA group II, in which *Burkholderia* and *Ralstonia* are allocated. The various symptoms produced in plants by the phytopathogenic pseudomonads are tumorous outgrowth, rot, blight or chlorosis, and necrosis, which are caused by alteration of the normal metabolism of plant cells by pathogenicity factors excreted by the bacteria. These factors include enzymes capable of degrading components of plant tissues, toxins, and plant hormones.

The phytopathogenic species of the genus *Burkholderia* mainly produce rots, due to active pectinolytic enzymes and cellulases (Gehring, 1962; Hildebrand, personal communication). Further details on symptoms and on the list of hosts attacked by each species will be given in the section of species descriptions at the end of this chapter.

Aside from acting as agents of diseases, members of the genus also participate in producing beneficial effects by antagonizing other phytopathogenic organisms, mainly fungi. This effect has been reported in the literature for *B. cepacia* in a number of instances (Kawamoto and Lorbeer, 1976; Fantino and Bazzi, 1982; Janisiewicz and Roitman, 1988; Homma et al., 1989; Jayaswal et al., 1990; Parke et al., 1991).

Pathogenicity for humans and animals The most serious animal and human pathogenic species of the genus *Burkholderia* are *B. mallei* and *B. pseudomallei*, the agents of glanders or farcy of equids, and of melioidosis in humans, respectively. Detailed descriptions of these organisms and the diseases that they cause are available (Redfearn et al., 1966; Redfearn and Palleroni,

1975). As a free-living species present in the warm regions of the planet, *B. pseudomallei* has not spread far from the equator, although in China it has reached a northern latitude of at least 25.5 degrees (Yang et al., 1995b).

A useful updated treatment of the bacteriology of glanders and melioidosis is available (Pitt, 1998). Many years ago, melioidosis was recognized as a glanders-like disease (Whitmore, 1913), and subsequently the organism was assigned to several different genera. In fact, this has also been true of *B. mallei*, which has spent much of its scientific career in search of a proper generic allocation.

Two different biotypes have been recognized among various strains of *B. pseudomallei* isolated from patients and from soil in Thailand. However, all of the strains were recognized by using a specific polyclonal antibody. One of the biotypes may have low virulence or it may represent a different species altogether, based on the distribution of these phenotypes and the respective incidence of melioidosis in different areas (Wuthiekanun et al., 1996).

Strains of *B. pseudomallei* isolated in Australia were examined for their genomic relationships using random amplification of polymorphic DNA and multilocus enzyme electrophoresis. The strains could be divided into two groups that correlated with the clinical presentation and not with the geographic origin; in other words, there was a correlation between the clinical manifestation of the disease and the molecular characteristics of the pathogen (Norton et al., 1998).

Some of the human pathogens are "opportunistic pathogens" that create major medical problems in patients with reduced levels of natural resistance. This situation emerges because of an increasing use of instrumentation or drugs (including antibiotics) that reduce or bypass the level of natural resistance and/or the specific immune mechanisms (Spaulding, 1974).

In reference to *B. cepacia*, it seems appropriate here to highlight the main points of the abstract of an excellent article published more than a decade ago (Goldmann and Klinger, 1986). From its original habitat as a plant pathogen, *B. cepacia* has invaded the hospital environment as an important pathogen of compromised human hosts. Many nosocomial infections have their source in contaminated medicaments and even disinfectants and antiseptics. Various conditions in hospital patients can be complicated by *B. cepacia* infections, but the properties that define the virulence of strains of this species are still poorly defined. The last addition to the long list of pathological conditions that are further deteriorated by infections of this pathogen is cystic fibrosis. As in other conditions, some patients may be simply colonized, while other patients' initial conditions can be very seriously complicated, to which the remarkable resistance of *B. cepacia* to a wide range of antibiotics contributes very effectively. This bleak panorama has become even worse in the intervening years, particularly with respect to pulmonary exacerbations in cystic fibrosis patients, in many cases ending in rapid and fatal deterioration of lung function.

Characteristics that occur more frequently among strains isolated from infections in cystic fibrosis patients include catalase, ornithine decarboxylase, valine amino-peptidase, lipase, alginase, trypsin, ability to reduce nitrate, hydrolysis of xanthine and urea, complete hemolysis of bovine red blood cells, cold-sensitive hemolysis of human red blood cells, and greening of horse and rabbit red blood cells. Although some of these properties are associated with pathogenicity in other bacteria, their relationships to cystic fibrosis-associated pulmonary disease are far from

clear (Gessner and Mortensen, 1990). Acid phosphatase in *Burkholderia* species is a factor that may be related to pathogenicity. It is present in *B. cepacia* and *B. pseudomallei*, and in fact the activity in the latter species is remarkably high (Dejsirilert Butraporn et al., 1989).

In addition to these factors, as mentioned before in the section on antigenic structure, LPS preparations from clinical and environmental isolates of *B. cepacia* and from the closely related species *B. gladioli* exhibit a higher endotoxic activity and more pronounced cytokine response *in vitro* when compared to preparations of *P. aeruginosa* LPS. The latter species is also involved in infections in cystic fibrosis patients (Shaw et al., 1995).

B. gladioli, a species originally described as phytopathogenic, has been implicated in infections complicating cases of cystic fibrosis (Mortensen et al., 1988). In one study, the organism was isolated from respiratory tract specimens obtained from 11 cystic fibrosis patients and was identified by its biochemical properties, DNA hybridization, and fatty acid analysis. The authors recommend the inclusion of some of these criteria for the differentiation between *B. gladioli* and *B. cepacia*, two closely related species, and point out that most *B. gladioli* strains have $C_{10:0\ 3OH}$ fatty acid, which is absent from *B. cepacia* lipids (Christenson et al., 1989). Also based on the results of fatty acid analysis, a pseudomonad that was isolated from pleural fluid and pulmonary decortication tissue with granulomatous disease more closely resembled *B. gladioli* than *B. cepacia* (Trotter et al., 1990). Further information on the activity of *B. gladioli* as a human pathogen has been reported by Ross et al. (1995). A strain of this species was involved in empyema and bloodstream infection occurring after lung transplantation in a cystic fibrosis case (Khan et al., 1996c).

The pili of *B. cepacia* mediate adherence to mucous glycoproteins (Kuehn et al., 1992; Sajjan and Forstner, 1993) and epithelial cells in cystic fibrosis patients. Structural variant classes of pilus fibers have been identified in *B. cepacia* strains (Goldstein et al., 1995). One or more of five morphologically different types can be coexpressed. It has been noticed that, when present in the infective population, *Pseudomonas aeruginosa* cells enhance the adhesion of *B. cepacia* to epithelial cells (Saiman et al., 1990).

The major pilin subunit of *B. cepacia* corresponds to peritrichous fimbriae, which, based on their appearance as giant intertwined fibers, have been called "cable" (Cbl) pili. The *cblA* gene (the first pilin subunit gene to be identified in *B. cepacia*) has been detected in a DNA library (Sajjan et al., 1995).

One very infectious cystic fibrosis strain isolated as the agent of epidemics in England and Canada was found to have the cable pilin subunit gene. A conserved DNA marker in a 1.4-Kb fragment was present in epidemic strains, absent from the nonepidemic ones, and rare among the environmental strains. The presumed ORF was designated "epidemic marker regulator" (*esmR*) (Mahenthiralingam et al., 1997). Strains were recovered in cystic fibrosis centers in France. There was cross-colonization in 7 of 13 centers. The most chronically colonized patients harbored a single *B. cepacia* strain, which suggests a geographically clustered distribution of *B. cepacia*, with the exception of one genotype. This genotype was detected in four regions and proved to be different from the British-Canadian highly transmissible strain and was able to spread among cystic fibrosis units (Segonds et al., 1997).

Biochemical and genomic properties have been used for the typing of *B. cepacia* strains of nosocomial origin. In a first step, six enzymes and pigment production subdivided a collection of

strains of this species into 12 groups, and the strains from one-third of the collection were further characterized by DNA fingerprinting, ribotyping, and plasmid analysis. By testing the typing scheme on strains isolated years later, the results showed the usefulness and consistency of the genomic typing, and the marked diversity of phenotypes among the strains of the species (Ouchi et al., 1995).

The amplified products of the internal transcribed spacers (ITS) separating the 16S and 23S rRNA genes in the DNA have been effective as tools for identification of reference strains of *Pseudomonas aeruginosa* and *B. pseudomallei*, but the primer pairs tested for *B. cepacia* have not provided much help in strain identification (Tyler et al., 1995). In contrast, PCR-ribotyping targeted on the 16S-23S intergenic spacer to determine the length heterogeneity of this region is a rapid and accurate method for *B. cepacia* strain typing (Dasen et al., 1994).

The internal diversity of *B. cepacia* strains is also manifested in differences in whole-cell protein profiles (Li and Hayward, 1994) and in the fatty acid composition of populations from various cystic fibrosis centers (Mukwaya and Welch, 1989).

A novel marker has been found to be associated with epidemic *B. cepacia* strains causing infections in cystic fibrosis patients. A highly infectious strain had both the cable pilin subunit gene (*cblA*) and a unique combination of insertion sequences. Although no specific marker was identified in common with other epidemic strains, a conserved DNA fragment among epidemic strains was detected. This fragment (called the "*B. cepacia* epidemic strain marker") was absent from other strains infecting individual cystic fibrosis patients and rarely found in environmental strains (Mahenthiralingam et al., 1997).

In a related study on 97 clinical and 2 environmental strains of *B. cepacia*, a search for possible correlations was made with respect to parameters such as certain insertion sequence (IS) elements, the *cblA* gene for a pilin subunit, the electrophoretic type (ET), and the ribotype (RT) (Tyler et al., 1996). No linkage was found between the presence of each of five different IS elements and ET or RT. All strains of a given ET also possessed *cblA*. One IS element different from the five initially identified was present in 72% of all isolates, and in half of them the new IS element was inserted in one of the original five. This hybrid IS element only was found in epidemic strains from Ontario, Canada, and the UK.

Methods were developed to detect the presence of *B. cepacia*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* in sputum, using primers based on 16S rRNA sequences. The results are in agreement with those of direct isolation of cultures from the samples (Campbell et al., 1995; Karpati and Jonasson, 1996).

Bongkreik acid (also named flavotoxin A), a product of fermentation by *B. cocovenenans*, is the cause of serious food poisoning outbreaks in China and other Far East countries (Hu et al., 1989). An isomer, isobongkreik acid, was later identified, and, like bongkreik acid, it acts as an uncompetitive inhibitor of ADP transport in mitochondria, although it is less active than bongkreik acid (Lauquin et al., 1976).

Ecology, habitats, and niches The habitats from which *Burkholderia* species have been isolated are quite diverse: soils and rhizospheres (*B. cepacia, B. vietnamiensis, B. pseudomallei, B. pyrrocinia, B. phenazinium, B. multivorans, B. thailandensis, B. glathei*), water (*B. norimbergensis*), plants (*B. cepacia, B. gladioli, B. caryophylli, B. glumae, B. plantarii, B. andropogonis, B. vandii*), foods (*B. cocovenenans*), animals (*B. mallei, B. pseudomallei*), and clinical

specimens (*B. cepacia, B. vietnamiensis, B. gladioli, B. multivorans*). The occurrence of *B. pseudomallei* mainly in soils of tropical regions has been discussed elsewhere (Redfearn et al., 1966).

As mentioned in the section on metabolic properties, some species of the genus are metabolically very versatile, and there is little doubt that those normally found in soils and water must contribute substantially to the mineralization of carbon compounds in nature.

B. cepacia is commonly found in natural materials, particularly soil, and methods have been developed for its detection. One such method of detection in the environment was based on amplification of sequences in genomic DNA using primers specific for repetitive extragenic palindromic segments, followed by cloning of the amplified fragments. Probes were constructed based on the strain-specific sequences (Matheson et al., 1997). A striking example of the power of molecular approaches was the report of the detection of a single *B. cepacia* cell in a soil sample containing a population of 10^{10} procaryotic cells (Steffan and Atlas, 1988). Estimates of the relative abundance of *B. cepacia* in stream bacterioplankton can be performed after collecting the cells by various procedures, of which the use of filters made of inorganic materials was found to give the highest recoveries (Lemke et al., 1997).

Strains of *B. cepacia* can be isolated from rhizosphere environments (Tabacchioni et al., 1995). This is also the habitat from which *B. graminis* was originally obtained (Viallard et al., 1998). Rhizosphere strains of *B. cepacia* differed in phenotypic characteristics from those isolated from clinical materials. Among the differences were nitrogen fixation, indole-acetic acid production, a wide temperature range, antibiosis vs. phytopathogenic fungi, and growth promotion of *Cucumis sativus*—all positive for rhizosphere strains. These properties were absent from clinical strains, which instead possessed characteristics such as adhesion to human cells, protease production, and synthesis of siderophores different from those found in the nonclinical strains (Bevivino et al., 1994). The character of N_2 fixation is not clear-cut, since DNA preparations from clinical strains hybridized with the *nifA* gene probe from *Klebsiella pneumoniae*, and the DNA of one of the rhizosphere strains hybridized with the *nifHDK* from *Azospirillum brasilense* (Tabacchioni et al., 1995).

As far as animal pathogenicity is concerned, observations made in other laboratories seem to support somewhat different conclusions. Thus, strains isolated from plant or clinical materials did not differ in their lethality to mice, i.e., they have similar LD_{50} values (González and Vidaver, 1979). Indeed, strains of species known to be causal agents of human infections may be isolated from rhizosphere environments (Tabacchioni et al., 1995).

Although *B. cepacia* helps in the degradation of toxic compounds and has an inhibitory action on soil-borne plant pathogens, it can also behave as a serious opportunistic human pathogen. The key question of interest for its use in biotechnological projects is whether the two types of populations can interact to the point of transmission of the characteristics required for the pathogenic condition. Yohalem and Lorbeer (1994) state that "although there are (*B. cepacia*) strains with significant potential for the remediation of environmental toxins . . . and others with potential as biological controls of plant disease . . . environmental release of any strain may be prohibited because some strains of the nomenspecies have been implicated in nosocomial infections." The same considerations apply to other versatile species of the genus that manifest pathogenic propensities.

ENRICHMENT AND ISOLATION PROCEDURES

For the specific isolation of *B. cepacia* from environmental water samples or various aqueous solutions, a medium containing 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan (C-390) and polymyxin B sulfate (PBS) has been proposed.[3] The two drugs inhibited all the organisms tested, with the exception of *B. cepacia* and *Serratia marcescens* (Wu and Thompson, 1984).

A medium proposed for the isolation of *B. cepacia* contains tryptamine and azelaic acid as sole sources of nitrogen and carbon, respectively, in addition to the antifungal compound chlorothalonil (Diamond Shamrock Corp., Cleveland, Ohio) (Burbage and Sasser, 1982). Later on it was found that the effectiveness of this medium was rather low, and a different one, medium (TB-T)[4], based on the combined action of trypan blue (TB) and tetracycline (T) at a relatively low pH (5.5), was proposed. Using this medium, the efficiency of recovery was very variable among strains, and for some of them reached 76–86% (Hagedorn et al., 1987).

A medium for *B. cepacia* has been described in a patent (Lumsden and Sasser, 1986).

Three selective enrichment liquid media and four solid media were evaluated at two temperatures (35°C and room temperature) for their capacity of supporting growth of *B. pseudomallei* strains. Enrichment with trypticase soy broth with addition of 5 mg of crystal violet and 20 mg of colistin per liter and subculture in Ashdown medium[5] gave the highest recovery rates and the greatest suppression of other members of the soil microflora. The results were comparable at the two temperatures (room temperatures ranged between 20°C and 32°C).

DIFFERENTIATION OF THE GENUS *BURKHOLDERIA* FROM OTHER GENERA

Similarities between *Burkholderia* and other Gram-negative bacteria A sharp differentiation of members of *Burkholderia* and *Pseudomonas* is difficult because of many similarities between these organisms. In the course of biochemical studies carried out at Berkeley before the rRNA–DNA hybridization experiments showed that "*P. multivorans*" (*B. cepacia*) was not closely related to the fluorescent group, it was noticed that key steps of the metabolism of aromatic compounds were remarkably similar between the two groups. In the section on the metabolism of aromatic compounds, some comments have been made on the similarity of components of some of the enzymes involved in the degradation by distantly related organisms. In fact, many details of the metabolic constitution of the fluorescent pseudomonads are far closer to those found in some species of RNA similarity group II—in particular, *B. cepacia*—than to those characteristic

of RNA group V (*Stenotrophomonas* and *Xanthomonas*), which is closer to RNA group I. Such is the case of the enzymes codified by genes *bphB* and *bphC* in *P. putida* and *B. cepacia*, and the same is true for the gamma-carboxymuconolactone decarboxylases of these species. Fluorescent organisms and *B. cepacia* are able to synthesize salicylic acid, which acts both as a siderophore and also as the precursor of another siderophore found in both groups of organisms, pyochelin. Members of the fluorescent group and *B. pyrrocinia*, a species related to *B. cepacia*, produce the antifungal antibiotic pyrrolnitrin, and similarities also have been observed in the pilin antigenic structures of *P. aeruginosa* and *B. cepacia* (Saiman et al., 1989).

Similarities have also been observed between *B. cepacia* and bacteria of groups other than the aerobic pseudomonads. With respect to the production of chaperonins (so named for their relationship with eucaryotic chaperones), an evolutionary homolog of the protein involved in plants in the assembly of ribulose-bisphosphate carboxylase-oxygenase (the key enzyme in CO_2 fixation) was identified in *E. coli*. This protein, GroEL, is one of the chaperonins, a group widely present in procaryotes and organelles of procaryotic origin (chloroplasts and mitochondria) (Hemmingsen et al., 1988). Aside from *E. coli* and pseudomonads, chaperonins have been identified in species of *Legionella, Bacillus, Borrelia, Treponema, Mycobacterium,* and *Coxiella* (Kaijser, 1975; Houston et al., 1990). In work performed on the cloning and nucleotide sequencing of the *groE* operons of *P. aeruginosa* and *B. cepacia*, a high degree of similarity was found, which extended to the *E. coli groEL*. The level of similarity with the human protein was lower, but still considerable. Comparable results were obtained in the study of a second chaperonin, GroES (Jensen et al., 1995).

The above-cited findings on chaperonins indicate that the similarities between groups I and II (*Pseudomonas* and *Burkholderia*) in some cases go beyond the dispensable catabolic systems or the biosynthesis of secondary products.

Differentiation of *Burkholderia* from related genera The examples of similarities mentioned in the previous section show that a sharp differentiation of members of *Burkholderia* and *Pseudomonas* is difficult. Studies on DNA–DNA hybridization (Ballard et al., 1970) and on rRNA–DNA hybridization (Palleroni et al., 1973) demonstrated that species of the genus were members of RNA similarity group II. They were eventually allocated to a newly proposed genus under the name *Burkholderia* (Yabuuchi et al., 1992), but two of the species (*B. pickettii* and *B. solanacearum*) were later transferred to the new genus *Ralstonia*, and the criteria for the differentiation of this genus from *Burkholderia* were defined (Yabuuchi et al., 1995). Unfortunately, as in the case of the proposal of the genus *Burkholderia*, the published differential characteristics were limited to those of a single strain of each species, which makes it impossible to estimate the intraspecies diversity.

In a polyphasic study of *Burkholderia* species, Gillis et al. (1995) redefined this genus. Among the characteristics of differential value, these authors mention the presence of $C_{16:0\ 3OH}$ in the cellular fatty acid composition. Most of the properties given in the definition of the genus *Burkholderia* apply equally well to other genera of aerobic pseudomonads, and, as in the case of the genus *Pseudomonas*, the fatty acid composition and the 16S rRNA characteristics remain among the few useful differential criteria.

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3. Plate Count Agar (PCA, Difco Laboratories) is rehydrated according to the manufacturer's instructions. Aqueous stock solutions containing 0.1% C-390 or 7.5% PBS are prepared. One milliliter of each of these solutions is added to each liter of rehydrated PCA to reach a final concentration of 1 µg/ml of C-390 and 75 µg/ml PBS. The medium is autoclaved at 121°C for 15 min.

4. TB-T agar medium (g/l): glucose, 2.0; L-asparagine, 1; NaHCO₃, 1.0; KH₂PO₄, 0.5; MgSO₄·7H₂O, 0.1; trypan blue, 0.05; and agar, 20.0. The pH is adjusted to 5.5 with phosphoric acid solution (4 ml/l of a 10% solution). After autoclaving, tetracycline (20 mg/l) is added from a filter-sterilized stock solution. When fungi are abundant in the sample, crystal violet (5 mg/l) and filter-sterilized nystatin (50 mg/l) are added.

5. Ashdown medium (Ashdown, 1979a, b) is trypticase soy agar (BBL) with the following additions (per liter): glycerol, 40 g; crystal violet, 5 mg; neutral red, 50 mg; and gentamicin, 4 mg.

TAXONOMIC COMMENTS

In the 1960s, a project that focused on the construction of a rational system of classification of *Pseudomonas* species was organized at the Department of Bacteriology of the University of California at Berkeley. This project had its main justification in the highly unsatisfactory situation of the taxonomy of this genus to which were assigned several hundreds of species names, many of which could not be identified based on published descriptions, and their type strains had been lost.

A thorough phenotypic characterization of a large collection of strains resulted in a subdivision of the genus into species and species groups (Stanier et al., 1966). With time, species not included in the original project were subjected to the same analysis and located in the classification scheme. Eventually, the phenotypic characterization received confirmation from the results of DNA–DNA hybridization experiments. More significantly, the results of these experiments revealed a very wide range of DNA similarity values among the species assigned to the genus, suggesting a considerable degree of genomic heterogeneity among the members of the genus. This hypothesis was corroborated by rRNA–DNA hybridization experiments. They clearly showed that *Pseudomonas*, as classically described, could be subdivided into five RNA similarity groups representing at least five different genera (Palleroni et al., 1973).

This demonstration that the genus *Pseudomonas* was in fact a complex entity of suprageneric hierarchy was taken as the basis for proposals from various other laboratories to give different generic names to designate members of the five rRNA similarity groups. Of the five groups, rRNA group I retained the name *Pseudomonas* and the type species *P. aeruginosa* was originally proposed for the genus. For rRNA group II, the new genus name *Burkholderia* was introduced (Yabuuchi et al., 1992), comprising seven new combinations: *B. cepacia* (Palleroni and Holmes, 1981), *B. mallei* (Zopf, 1885), *B. pseudomallei* (Whitmore, 1913), *B. caryophylli* (Burkholder, 1942), *B. gladioli* (Severini, 1913), *B. pickettii* (Ralston et al., 1973), and *B. solanacearum* (Smith, 1896). Since the heterogeneity of the genus even extended to the rRNA similarity group level, some of the groups could still be further subdivided to include more than one bacterial genus. Thus, the last two of the above-mentioned species, were assigned to a newly created genus, *Ralstonia* (Yabuuchi et al., 1995). The overall similarity of *Ralstonia solanacearum* and *R. pickettii* already had been noticed in studies on their phenotypic characteristics and on DNA homologies (Ralston et al., 1973).

The present treatment refers to those species of rRNA group II assigned to the genus *Burkholderia*.

In their studies on the aerobic pseudomonads, Stanier et al. (1966) described a group of metabolically versatile organisms under the new species name *"Pseudomonas multivorans"*. In the plant pathology department of the University of California, David Sands applied the same methodology to the study of phytopathogenic pseudomonads. Soon his studies resulted in the unexpected finding that the properties of the above strains were virtually identical to those of a species that had been known to phytopathologists for almost two decades under the name of *P. cepacia*. The remarkable versatility of strains of this species had been totally overlooked by the early workers.

DNA–DNA hybridization experiments confirmed the synonymy soon thereafter (Ballard et al., 1970). The collection of strains of *B. cepacia* examined at the time could be differentiated from other species on the basis of the capacity to grow at the expense of D-arabinose, D-fucose, cellobiose, saccharate, mucate, 2,3-butylene glycol, sebacate, *meso*-tartrate, citraconate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, L-threonine, DL-ornithine, and tryptamine. This set of characteristics sharply separated *B. cepacia* from all other members of the various phenotypic groups, as can be seen in Table 52 of the original reference (Stanier et al., 1966).

The early observations that helped to uncover the remarkable metabolic versatility of *B. cepacia* and other species of the genus *Burkholderia* also contributed substantial lists of phenotypic characteristics for use in the identification of newly isolated strains. Some of the results are summarized in the tables included in this chapter. Substantial collections of strains of the species are now available, and studies on intraspecific diversity have been published. The interesting metabolic properties of members of this group of procaryotes stimulated the isolation of many strains, which has contributed to the recent proliferation of names for new species and for groups (biovars, genomovars) at the intraspecific level. On the one hand, the nutritional versatility of *Burkholderia* species—which includes the ability to degrade toxic environmental contaminants—suggests an important function in the carbon cycle in nature. However, research directed to the use of these characteristics in bioremediation projects has to take into account the fact that some of the species are serious pathogenic agents for plants and animals, and proper precautions should be taken to counteract these activities.

Early studies on *B. cepacia* and its synonym *"Pseudomonas multivorans"* (Stanier et al., 1966; Ballard et al., 1970) clearly indicated considerable intraspecific heterogeneity in phenotypic characteristics such as pigmentation and nutritional properties, and in the DNA similarity results of DNA–DNA hybridization experiments. One hundred strains of *B. cepacia* were examined, and a proposal for a subdivision of the species into biovars was suggested (Richard et al., 1981). In recent times, other comparative studies showed marked differences between *B. cepacia* strains isolated from the clinical environment and those found in plant rhizospheres (Bevivino et al., 1994; Tabacchioni et al., 1995). Excellent reviews are available on the intraspecific diversity of this species (Yohalem and Lorbeer, 1994) and on its genomic complexity and versatility (Lessie et al., 1996). All these reports have had a taxonomic impact, resulting in the proposal of converting some of the intraspecific groups into independent taxa at the species level, as discussed below.

The newly proposed species names include *B. vietnamiensis* and *B. multivorans*, related to *B. cepacia*, *B. thailandensis*, a relative of *B. pseudomallei*, and *B. graminis*, which is close to the group *B. caryophylli*–*B. glathei*–*B. phenazinium*. Some of the descriptions fail to create a precise circumscription of the proposed taxa, but it is to be expected that the interest in these organisms will eventually contribute to generate characterizations that are more comprehensive.

A genotypic analysis of 128 strains of *Burkholderia*, *Ralstonia*, and *Pseudomonas* was taken as the basis for a definition of the taxonomic structure of *B. cepacia* (Vandamme et al., 1997b). The strains isolated from cases of cystic fibrosis could be grouped into five so-called genomic species, very similar from the phenotypic standpoint. One of the genomic species of this "*B. cepacia* complex" corresponded to the previously described *B. vietnamiensis*, and a second one received the formal name *B. multivorans*, a revival of a synonym of *B. cepacia* used many years before (Stanier et al., 1966). The rest of the genomovars (I, III, and IV) have remained unnamed.

A description of *B. multivorans* is given in this chapter (see

List of Species of the genus *Burkholderia*), and a more complete report of its properties can be found in the original description (Vandamme et al., 1997b). Four of the strains of genomovar II— now *B. multivorans*—exhibited a high level of relatedness by DNA–DNA hybridization. The organisms are phenotypically similar to other biovars of *B. cepacia* and cannot be readily differentiated from them.

The name *B. thailandensis* was recently proposed for a group of strains closely related to *B. pseudomallei* based on a high 16S rRNA sequence similarity (Brett et al., 1998). The organisms differ phenotypically from *B. pseudomallei* by relatively few characteristics, including an ability to use L-arabinose, 5-ketogluconate, adonitol, erythritol, and dulcitol. Differences in biochemical profile and virulence also occur. DNA–DNA hybridization studies of the two species have not been reported.

The position occupied by *B. graminis* in the phylogenetic tree of Fig. BXII.β.2 indicates that this species is located in a cluster that includes *B. glathei* and *B. phenazinium*. This is further supported by a set of phenotypic properties of differential value and clear-cut differences in the DNA reassociation values, as can be seen in Tables 2 and 3 of the original publication (Viallard et al., 1998).

Finally, a new species with rather unique properties, *B. norimbergensis* (*Pandoraea norimbergensis*), has been described for a strain isolated from an oxic water layer above a sulfide-containing sediment (Wittke et al., 1997). This organism oxidizes several inorganic sulfur compounds including sulfur and produces sulfate. The reported characteristics of the new species do not fit the organization of the tables in this chapter. The description is given in the list of species for the genus *Pandoraea*, together with some comments on its phylogenetic relationships. The 16S rRNA sequence of a new species of *Burkholderia* that has been named *B. caribensis* has been deposited in GenBank under the accession numbers Y17009, Y17010, and Y17011. The sequence places this species near *B. graminis* (V. Viallard and J. Balandreau, personal communication).

Following the recommendation formulated by Vandamme et al. (1997b), buttressed by the evidence reported by Viallard et al. (1998), three species previously assigned to *Pseudomonas* are now being included in the list of *Burkholderia* species as *B. glathei*, *B. pyrrocinia*, and *B. phenazinium*.

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FIGURE BXII.β.2. Neighbor-joining tree obtained with the 16S rDNA sequences of *Burkholderia*, [*Pseudomonas*], and *Ralstonia* species. Bootstrap values (1000 resamplings) greater than 50% are indicated at the nodes, and asterisks indicate values higher than 50% found in the parsimony analysis. Bar = 0.0073 fixed mutations per nucleotide position. Three additional strains of *B. vietnamiensis* had the same sequence as the type strain represented in the tree. The tree shows the position of several unnamed strains whose source is indicated in the original paper (Viallard et al., 1998). Of these, strain E264 has been named recently *B. thailandensis* (Brett et al., 1997). [*P.*] *glathei*, [*P.*] *phenazinium*, and [*P.*] *pyrrocinia* are described in this chapter as *Burkholderia* species. (Reproduced with permission from V. Viallard et al., International Journal of Systematic Bacteriology 48: 549–563, 1998, ©International Union of Microbiological Societies.)

List of species of the genus Burkholderia

1. **Burkholderia cepacia** (Palleroni and Holmes 1981) Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398^VP (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1271) (*Pseudomonas cepacia* Palleroni and Holmes 1981, 479.)

ce.pa' ci.a. L. fem. n. *caepa* or *cepa* onion; M.L. fem. adj. *cepacia* of or like an onion.

Properties useful for differentiation from other species of the genus are given in Table BXII.β.2. Characteristics of the species are presented in Table BXII.β.3. As suggested in the description of *"Pseudomonas multivorans"* (Stanier et

al., 1966), strains of *B. cepacia* can be differentiated from other species on the basis of their ability to grow at the expense of D-arabinose, D-fucose, cellobiose, saccharate, mucate, 2,3-butylene glycol, sebacate, *m*-tartrate, citraconate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, L-threonine, DL-ornithine, and tryptamine. These characteristics, although not present in all strains, are useful for the identification of newly isolated strains. For further descriptive information see Ballard et al. (1970) and Palleroni and Holmes (1981). Optimal growth temperature, ~30–35°C.

Many strains have been isolated from rotten onions, soils, various natural materials, and clinical specimens. The species is considered a serious opportunistic human pathogen, and it has been found associated with infections of nosocomial origin. A study by Vandamme et al. (1997b) on strains isolated from cystic fibrosis cases has resulted in the identification of multiple genomovars of *B. cepacia*, among which some (genomovar II) are being described under the new species name *Burkholderia multivorans* (see comments in the description of this species below).

The mol% G + C of the DNA is: 67.4 (Bd).

Type strain: ATCC 25416, Ballard 717, DSM 7288, ICPB 25, NCTC 10743.

GenBank accession number (16S rRNA): M22518, U96927.

2. **Burkholderia andropogonis** (Smith 1911) Gillis, Van, Bardin, Goor, Hebbar, Willems, Segers, Kersters, Heulin and Fernandez 1995, 287^VP (*Pseudomonas andropogonis* (Smith 1911) Stapp 1928, 27; *Pseudomonas andropogoni* (sic) Smith 1911, 63.)

an.dro.po′go.nis. M.L. n. *Andropogon* genus of widely distributed grasses; M.L. gen. n. *andropogonis* of the genus *Andropogon.*

The following description is slightly modified from the one given by Palleroni (1984) in the first edition of this *Manual.*

Slender rods with rounded ends, 0.5–0.7 × 1–2 µm, with one or rarely two polar flagella. Colonies change from butyrous to viscid with age. No fluorescent pigment is produced. Most strains are oxidase negative. Gelatin liquefaction, nitrate reduction, lipolysis, and arginine dihydrolase reactions negative. Production of sheathed flagella (flagella surrounded by a membrane that is a continuation of the cell wall) was reported by Fuerst and Hayward (1969a).

Although the species had been tentatively assigned to the rRNA similarity group III based on its enzymatic properties, it is now considered a member of the genus *Burkholderia* (Gillis et al., 1995). Further details of its phenotypic properties are given by Palleroni (1984) and in Tables BXII.β.2 and BXII.β.3. A comprehensive description is given in the Ph.D. thesis of Xiang Li (1993).

Recommendations to consider *P. stizolobii* and *P. woodsii* as synonyms of *B. andropogonis* were formulated by several workers (Goto and Starr, 1971; Hayward, 1972; Nishiyama et al., 1979; Shanks and Hale, 1984), and this opinion was reinforced by the demonstration of similar protein profiles (Vidaver and Carlson, 1978) and polyamine composition (Auling et al., 1991).

Pathogenic for sorghum, corn, clover, and velvet bean (*Stizolobium deeringianum*). To the host list should be added highbush blueberry, in which the pathogen causes leaf spot lesions in hardwood cuttings (Kobayashi et al., 1995). Perhaps the host specialization may justify creating two pathovars—pathovar *andropogonis* for the causal agent of the bacterial stripe of sorghum, and pathovar *stizolobii* for that of the bacterial leaf spot of velvet bean (Palleroni, 1984). As mentioned in a previous section, strains of *B. andropogonis* are able to synthesize rhizobitoxine, a phytotoxin capable of causing foliar chlorosis.

The mol% G + C of the DNA is: 59–61.3 (T_m).

Type strain: ATCC 23061, DSM 9511, LMG 2129.

GenBank accession number (16S rRNA): X67037.

3. **Burkholderia caribensis** Achouak, Christen, Barakat, Martel and Heulin 1999, 792^VP

ca.ri.ben′ sis. M.L. adj. *caribensis* pertaining to the Caribbean Islands, where the strains were isolated.

The description is taken from the original paper.

Short rods, 1–2 × 0.5 µm. Motile and pleomorphic in actively growing cultures in LB medium. In sugar-rich media (with 2% glucose, xylose, fructose, sorbitol, arabinose, mannitol, or sorbitol) it produces abundant exopolysaccharide.

Oxidase, catalase, urease, arginine dihydrolase, and β-galactosidase are produced. A list of organic compounds that have been tested as substrates of oxidative activities is given in the paper. Isolated from a vertisol fraction in the island of Martinique.

The mol% G + C of the DNA is: 63.1 (T_m).

Type strain: LMG 18531, MWAP 64.

GenBank accession number (16S rRNA): Yl7009.

4. **Burkholderia caryophylli** (Burkholder 1942) Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398^VP (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1273) (*Pseudomonas caryophylli* (Burkholder 1942) Starr and Burkholder 1942, 601; *Phytomonas caryophylli* Burkholder 1942, 143.)

ca.ry.o′phyl.li. M.L. masc. n. *caryophyllus* specific epithet of *Dianthus caryophyllus*, carnation; M.L. gen. n. *caryophylli* of the carnation.

The general characteristics of the species are presented in Tables BXII.β.2, BXII.β.3, and BXII.β.6. The main properties for differentiation from several other *Burkholderia* species and other denitrifying pseudomonads, are summarized in Table BXII.β.4. Optimal growth temperature ~30–33°C. For further descriptive information, see Ballard et al. (1970). Isolated from diseased carnations.

The mol% G + C of the DNA is: 65.3 (Bd).

Type strain: ATCC 25418, DSM 50341, ICPB PC113, NCPPB 2151, PDDCC 512.

GenBank accession number (16S rRNA): X67039.

5. **Burkholderia cocovenenans** (van Damme, Johannes, Cox and Berends 1960) Zhao, Qu, Wang and Chen 1995, 601^VP (*Pseudomonas cocovenenans* van Damme, Johannes, Cox and Berends 1960, 255.)

co.co.ve′ ne.nans. M.L. n. *Cocos* genus of coconut; L. v. *veneno* to poison; M.L. part. adj. *cocovenenans* coconut poisoning.

The following description is taken from the paper by Zhao et al. (1995). Rods 0.3–0.5 × 1.6–2.0 µm, occurring singly or in pairs. Motile by means of one to five polar flagella. No lipid soluble or fluorescent pigment is produced; however, a greenish-yellow diffusible pigment (tox-

TABLE BXII.β.6. Nutritional characteristics of *Burkholderia cepacia*, *Burkholderia gladioli*, and *Burkholderia caryophylli*[a]

Characteristics	*B. cepacia*	*B. gladioli*	*B. caryophylli*
Utilization of:			
D-Ribose, D-arabinose, L-arabinose, D-fucose, D-glucose, *N*-acetylglucosamine[b], D-mannose, D-galactose, D-fructose, sucrose, cellobiose, gluconate, 2-ketogluconate, sacchare, mucate, salicin, acetate, propionate, butyrate, isobutyrate, valerate, malonate, succinate, fumarate, D-malate, L-malate, *m*-tartrate, β-hydroxybutyrate, lactate, glycerate, hydroxymethyl-glutarate, citrate, α-ketoglutarate, pyruvate, aconitate, mannitol, sorbitol, *m*-inositol, adonitol[b], glycerol, *p*-hydroxybenzoate, phenylacetate, quinate, L-alanine, β-alanine, L-serine, L-cysteine[b], L-aspartate, L-glutamate, L-arginine, γ-aminobutyrate, L-histidine, L-proline, L-tyrosine, L-phenylalanine, L-tryptophan, betaine, hippurate	+	+	+
D-Xylose, *n*-propanol	d	+	+
L-Rhamnose, glycolate	d	–	+
Trehalose, L-threonine	+	+	d
Isovalerate, glutarate, citrulline, anthranilate, sarcosine	+	d	–
Heptanoate, caproate, caprylate, pelargonate, caprate, adipate, azelate, sebacate, citraconate, adonitol, dulcitol[b], benzoate, D-alanine, ornithine, kynurenate, ethanolamine, 2-aminobenzoate	+	+	–
Lyxose[b], tagatose[b], 5-ketogluconate[b], melibiose[b], gentiobiose[b], raffinose[b], amygdalin[b], arbutin[b], aesculin[b], salicin[b], L-arabitol[b], pimelate, suberate, levulinate, *m*-hydroxybenzoate, δ-aminovalerate, putrescine, spermine, butylamine, tryptamine, α-amylamine, diaminobutane[b]	+	–	
D(−)-Tartrate, mesaconate	–	+	–
L(+)-Tartrate, ethanol, L-isoleucine, nicotinate, trigonelline	d	+	–
Itaconate, propylene glycol, glycine, norleucine, α-aminobutyrate, α-aminovalerate	–	d	–
L-Fucose[b], 2,3-butylene glycol, D-arabitol[b], xylitol[b]	+	–	+
n-Butanol	d	d	+
Isobutanol	d	d	–
L-Mandelate, benzoylformate	d	–	d
o-Hydroxybenzoate, testosterone, benzylamine, histamine, acetamide	d	–	–
L-Leucine, L-valine	d	+	d
Dodecane, hexadecane	d		

[a]For symbols see standard definitions.

[b]Data for the type strains, taken from Yabuuchi et al. (1992).

oflavin) is formed after 1–2 d of incubation on potato dextrose agar or other media.

Growth temperature range goes from 6–41°C. Metabolism is respiratory and denitrification is negative, but nitrate is reduced to nitrite. Oxidase reaction is negative. Gelatinase, Tween 80 hydrolysis, and lecithinase reactions are all positive. Many organic compounds can be used as sole carbon and energy sources. Detailed information can be found in the original report (Zhao et al., 1995), in Gillis et al. (1995), and in Tables BXII.β.2 and BXII.β.3.

Strains have been isolated from fermented coconut food (bongkrek) in Indonesia, fermented cornmeal in China, deteriorated white fungus (*Tremella fuciformis*), and soil. DNA–DNA hybridization experiments have shown that this species is close to *B. cepacia* and *B. gladioli* (Zhao et al., 1995). The organism is not infectious to humans, but it is responsible for serious cases of food poisoning due to the production of a yellow poisonous compound, toxoflavin.

Recent studies by Coenye et al. (1999b) based on whole protein electrophoretic profiles, DNA–DNA hybridization, and comparison of many biochemical properties indicate that *B. cocovenenans* should be considered a junior synonym of *B. gladioli*.

The mol% G + C of the DNA is: 69 ± 0.5 (T_m).

Type strain: ATCC 33664, DSM 11318, LMG 11626, NCIB 9450.

GenBank accession number (16S rRNA): U96934.

6. **Burkholderia gladioli** (Severini 1913) Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398[VP] (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1273) (*Pseudomonas gladioli* Severini 1913, 420.)

gla.di.o′li. L. n. *gladiolus* a small sword lily; M.L. masc. n. *gladioli* of gladiolus.

The characteristics of the species are summarized in Tables BXII.β.2, BXII.β.3, and BXII.β.6. Optimal growth temperature, ∼30–35°C. Further descriptive information may be found in Ballard et al. (1970), where the species appears under the name *"Pseudomonas marginata"* (see also Hildebrand et al., 1973).

Isolated from decayed onions, *Gladiolus* spp, and *Iris* spp., for which the species is believed to be pathogenic. Two pathovar names have been proposed for the phytopathogenic strains, pathovar *gladioli* and pathovar *aliicola* (Young et al., 1978). To these, the new pathovar name pathovar *agaricicola* has been created to distinguish the *B. gladioli* strains producing rapid soft rot of cultivated mushrooms (*Agaricus bitorquis*) (Lincoln et al., 1991). *B. gladioli* was identified as the agent of leaf spot and blight of the bird's nest fern *Asplenium nidus* (Chase et al., 1984). As indicated in the section on pathogenicity for humans, strains of the species have been found to be serious opportunistic pathogens.

As a phytopathogen, *B. gladioli* pathovar *alliicola* behaves in a manner similar to that of *B. cepacia*, causing soft rot of onions (61-gl; 21-gl) (Tesoriero et al., 1982; Wright et al., 1993). It has also been identified as a probable agent of early blight in cherries (26-gl) (Li and Scholberg, 1992), and to cause rapid soft rot disease of the edible mushroom *Agaricus bitorquis* (38-gl; 29-gl) (Lincoln et al., 1991; Atkey et al., 1992). *B. gladioli* also contributes to postharvest dis-

TABLE BXII.β.7. Nutritional properties of *Burkholderia mallei* and *Burkholderia pseudomallei* [a]

Characteristics[b]	B. mallei	B. pseudomallei
Utilization of:		
Acetate, *N*-acetylglucosamine[c], adipate, D-alanine, L-alanine, β-alanine, γ-aminobutyrate, D-arabinose, L-arginine, L-aspartate, benzoate, betaine, cellobiose, D-fucose, fumarate, galactose, glucose, L-glutamate, glycerate, glycerol, hippurate, L-histamine, β-hydroxybutyrate, *p*-hydroxybenzoate, *m*-inositol, α-ketoglutarate, lactate, L-malate, mannitol, mannose, phenylacetate, poly-β-hydroxybutyrate, propionate, pyruvate, quinate, sorbitol, succinate, sucrose, L-threonine, trehalose, tryptamine, L-tyrosine	+	+
Aconitate, 2-aminobenzoate[c] α-amylamine, D-arabitol[c], benzoylformate, butylamine, caprate, caproate, L-cysteine[c], dulcitol[c], erythritol, ethanol, ethanolamine, L-fucose[c], glycogen[c], heptanoate, isobutyrate, L-isoleucine, isovalerate, kynurenate, kynurenine, levulinate, L-lysine, pelargonate, putrescine, ribose, sebacate, valerate	−	+
Glycine, D-xylose	+	−
α-Aminobutyrate, L-arabinose, malonate	d	−
δ-Aminovalerate, anthranilate, butyrate, caprylate, citrate, fructose, gluconate, 2-ketogluconate, maltose, L-phenylalanine, L-proline, putrescine, salicin, L-serine, suberate, starch, L-valine	d	+
Azelate, glutarate, D-malate, pimelate, sarcosine, trigonelline	d	d
Adonitol, ethanol, hexadecane, L-mandelate, spermine	−	d

[a]For symbols see standard definitions.

[b]The following compounds are not used by either species: acetamide, aesculin[c], *m*-aminobenzoate, *p*-aminobenzoate, α-amino-valerate, amygdalin[c], arbutin[c], benzylamine, *n*-butanol, 2,3-butylene glycol, citraconate, L-citrulline, creatine, dodecane, ethylene glycol, gentiobiose[c], geraniol, glycolate, histamine, *m*-hydroxybenzoate, *o*-hydroxybenzoate, hydroxymethylglutarate, inulin, iso-butanol, isophthalate, isopropanol, 5-ketogluconate, lactose, L-leucine, lyxose[c], maleate, D-mandelate, melibiose[c], mesaconate, methanol, methylamine[c], mucate, nicotinate, norleucine, L-ornithine, oxalate[c], pantothenate, phenol, phenylethanediol, phthalate, *n*-propanol, propylene glycol, raffinose[c], L-rhamnose, saccharate, salicin[c], tagatose[c], D(−)-tartrate, L(+)-tartrate, *meso*-tartrate, terephthalate, testosterone, tryptamine, D-tryptophan, xylitol[c].

[c]Data for the type strains, taken from Yabuuchi et al. (1992).

eases of fruits and vegetables, an activity in which the species has been identified by analyses of fatty acid composition (24-gl) (Wells et al., 1993).

The mol% G + C of the DNA is: 68.5 (Bd).

Type strain: ATCC 10248, DSM 4285, NCPPB 1891, PDDCC 3950.

GenBank accession number (16S rRNA): X67038.

Additional Remarks: This is also the reference strain for *B. gladioli* pathovar *gladioli*. The reference strain for *B. gladioli* pathovar *alliicola* is ATCC 19302 (PDDCC 2804; NCPPB 947). The reference strain for *B. gladioli* pathovar *agaricicola* is NCPPB 3580.

7. **Burkholderia glathei** (Zolg and Ottow 1975) Vandamme, Holmes, Vancanneyt, Coenye, Hoste, Coopman, Revets, Lauwers, Gillis, Kersters and Govan 1997b, 1199[VP] (*Pseudomonas glathei* Zolg and Ottow 1975, 296.)

gla′the.i. M.L. gen. *glathei* of Glathe, named after H. Glathe of Giessen, Germany.

The description is taken from Zolg and Ottow (1975). Rods to oval cocci, 0.5–0.7 × 1.5 μm, motile by a polar flagellum. Optimal growth temperature 30–37°C. Oxidase reaction positive. Negative for hydrolysis of starch, gelatin, lecithin (egg yolk reaction), esculin, and polypectate. Tributyrin, urea, and hippurate are hydrolyzed. Nitrate is reduced to nitrite. Denitrification and H$_2$S production are negative. No growth factor requirement has been found. The organism is capable of growth in nitrogen-deficient media, but acetylene reduction by cells grown under those conditions is negative. Acid tolerant (pH 4.5).

From the extensive phenotypic characterization of *B. glathei* following the methodology described by Stanier et al. (1966), it has been found that at least 68 organic com-

pounds can be utilized as sole carbon and energy sources for growth. These include aldoses, ketoses, deoxysugars, sugar-alcohols, and sugar-acids. Except for lactose, melibiose, and melezitose, no di-, tri-, and polysaccharides are utilized. The only amino acids that are not utilized are glycine, L-serine, L-isoleucine, L-methionine, and β-alanine. Of 28 aliphatic organic acids, 23 are utilized. The list of utilizable acids includes oxalate. Some properties are summarized in Table BXII.β.3.

A 1,2-oxygenase responsible for the *ortho* cleavage of aromatic compounds is produced constitutively. This species differs in several important characteristics from other species of the genus. The main differences include the capacity for growing in nitrogen-deficient media, the acid tolerance, and the utilization of oxalate for growth. A study by Vandamme et al. (1997b), which includes fatty acid analysis, indicates that this species should be allocated to the genus *Burkholderia*, an opinion that has found confirmation by rRNA sequencing studies (Viallard et al., 1998).

The mol% G + C of the DNA is: 64.8 (T$_m$).

Type strain: N15, ATCC 29195, DSM 50014, LMG 14190.

GenBank accession number (16S rRNA): U96935, Y17052.

8. **Burkholderia glumae** (Kurita and Tabei 1967) Urakami, Ito-Yoshida, Araki, Kijima, Suzuki and Komagata 1994, 242[VP] (*Pseudomonas glumae* Kurita and Tabei 1967, 111.)

glu′mae. L. n. *gluma* hull; L. gen. n. *glumae* of a husk.

The following description is taken from the original paper by Kurita and Tabei (1967). Rods, 0.5–0.7 × 1.5–2.5 μm, motile by means of two to four flagella. A fluorescent pigment is produced in potato agar. Nitrate reduction, starch hydrolysis, and H$_2$S production are negative. Gelatin liquefaction was not reported in the original paper, but Ura-

kami et al. (1994) found that the test was positive. Temperature limits for growth: 11–40°C; optimum: 30–35°C. Acid is produced from arabinose, glucose, fructose, galactose, mannose, xylose, glycerol, mannitol, and inositol. No acid is produced from rhamnose, sucrose, maltose, lactose, raffinose, dextrin, starch, inulin, or salicin. Milk is coagulated and peptonized. Pathogenic for the rice plant (*Oryza sativa*, fam. Gramineae).

To the above description, Urakami et al. (1994) have added a substantial amount of information on the nutritional spectrum of the type strain, and part of it has been summarized in Tables BXII.β.2 and BXII.β.3. A peculiar property of this species is the production of fluorescent pigment, but no further details are available on its relationship to the pigment characteristic of the fluorescent species of the genus *Pseudomonas*. This is a point of interest for further investigation. As mentioned before, two subgroups of *B. glumae* strains can be distinguished based on their fatty acid composition (Stead, 1992).

The mol% G + C of the DNA is: 68.2 (reversed HPLC) (Urakami et al., 1994).

Type strain: ATCC 33617, DSM 7169, LMG 2196, NCPPB 2981, NIAES 1169.

GenBank accession number (16S rRNA): U96931.

9. **Burkholderia graminis** Viallard, Poirier, Cournoyer, Haurat, Wiebkin, Ophel-Keller and Balandreau 1998, 560[VP]

gra′ mi.nis. M.L. adj. *graminis* referring to its isolation from the rhizosphere of grasses.

The description is taken from the original paper (Viallard et al., 1998). Rods, 0.3–0.8 × 1.0–1.5 μm; motile. The number of flagella per cell is not reported. Colonies on LB agar are thin, brownish-yellow, and translucent. On agar prepared with the special medium PCAT (Burbage and Sasser, 1982) after 3 d at 28°C, the colonies are white, somewhat opaque and creamy, with entire margin. Oxidase, catalase, urease, and arginine deiminase reactions are positive. Nitrates are reduced, but there is no denitrification. Characteristics in common with other *Burkholderia* species are the assimilation of glycerol, D- and L-arabinose, ribose, galactose, glucose, fructose, mannose, inositol, mannitol, sorbitol, D-arabitol, gluconate, and 2-ketogluconate, and the inability to use L-sorbose, methyl-α-D-xyloside, methyl-α-D-mannoside, methyl-α-D-glucoside, inulin, melezitose, starch, glycogen, or D-turanose. Properties not found in some of the other species include the incapacity of acid formation from glucose, to hydrolyze esculin or to produce gelatinase. In addition, the strains grow on L-xylose, lactose, rhamnose, trehalose, D-lyxose, L-arabitol, xylitol, and raffinose, but not on dulcitol or D-tagatose. Isolated from the rhizosphere of wheat, corn, and pasture grasses.

The mol% G + C of the DNA is: 62.5–63.0 (HPLC).

Type strain: C4D1M, ATCC 700544.

GenBank accession number (16S rRNA): U96939.

10. **Burkholderia kururiensis** Zhang, Hanada, Shigematsu, Shibuya, Kamagata, Kanagawa and Kurane 2000a, 747[VP]

ku.ru.ri.en′ sis. M.L. adj. *kururiensis* referring to Kururi, Chiba Prefecture, Japan, where the strain was isolated.

The following description is taken from the original paper. The cells are Gram negative and ovoid or rod shaped. (1 × 1.2–1.5 μm), occurring singly or in pairs. Growth occurs between 15–42°C, with an optimum at 37°C. The optimal growth pH is 7.2. Under optimal conditions, the doubling time is about 1 h. Oxidase and catalase positive. Starch and gelatin are not hydrolyzed, but glycogen and Tween 80 are. The following organic compounds are degraded aerobically: arabinose, fructose, fucose, galactose, glucose, lactulose, maltose, mannose, psicose, rhamnose, adonitol, arabitol, glycerol, inositol, mannitol, sorbitol, xylitol, N-acetylgalactosamine, acetate, citrate, formate, galacturonate, gluconate, lactate, propionate, alanine, asparagine, aspartate, glutamate, glycine, histidine, leucine, phenylalanine, proline, serine, threonine, inosine, 2,3-butanediol, benzene, *p*-cresol, fluorobenzene, and phenol. The following compounds are not oxidized: cellobiose, lactose, melibiose, raffinose, sucrose, trehalose, dextrin, malonate, uridine, thymidine, glucose-1-phosphate, and glucose-6-phosphate. UQ-8 is the dominant respiratory quinone. Main cellular fatty acids are $C_{16:0}$, cyclopropanic acids $C_{17:0}$, cyclopropanic acid $C_{19:0}$, $C_{16:1}$, and $C_{18:1}$. $C_{13:1}$ and $C_{17:1}$ are also present. The organism was isolated from an aquifer polluted with trichloroethylene (TCE) in Kururi, Chiba Prefecture, Japan, and shows degradation activity for this contaminant when the cells are grown in the presence of phenol.

The mol% G + C of the DNA is: 64.8 (HPLC).

Type strain: KP23, ATCC 700977, CIP 106643, DSM 13646, LMG 19447.

GenBank accession number (16S rRNA): AB024310.

This species has some properties that are not common in the genus *Burkholderia*. The cells are nonmotile, a characteristic shared only with *B. mallei*. The strain uses maltose, which is rarely used by strains of other species, and fails to use disaccharides used by the latter. No DNA–DNA hybridization data have been reported.

11. **Burkholderia mallei** (Zopf 1885) Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398[VP] (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1271) (*Pseudomonas mallei* (Zopf 1885) Redfearn, Palleroni and Stanier 1966, 305; *Bacillus mallei* Zopf 1885, 89.)

mal′ le.i. L. n. *malleus* the disease of glanders; L. gen. n. *mallei* of glanders.

Characteristics of the species are listed in Tables BXII.β.2, BXII.β.3, and BXII.β.7, and those that are useful for the differentiation of the species from various other denitrifying pseudomonads are summarized in Table BXII.β.4. Optimal growth temperature ~37°C. For further descriptive information, see Redfearn et al. (1966) and Redfearn and Palleroni (1975). Parasitic on horses and donkeys, in which it causes glanders and farcy. The infection is transmissible to humans and to other animal species.

The mol% G + C of the DNA is: 69 (Bd).

Type strain: NBL 7, ATCC 23344.

GenBank accession number (16S rRNA): S55000.

12. **Burkholderia multivorans** Vandamme, Holmes, Vancanneyt, Coenye, Hoste, Coopman, Revets, Lauwers, Gillis, Kersters and Govan 1997b, 1198[VP]

mul.ti′ vo.rans. L. adj. *multus* much; L. part. adj. *vorans* devouring, digesting; M.L. part. adj. *multivorans* digesting many compounds.

Rods, 0.6–0.9 × 1.0–2.0 µm. Motile at room temperature and at 37°C. Number of flagella per cell not reported. Able to grow at 42°C but not at 5°C. According to the original description, some strains grow at room temperature. No pigments are produced. Growth in some media used for enteric bacteria is reported. No information is given on utilization of carbon compounds for growth, but, instead, production of acids from some compounds is reported. Tolerance to cyanide is strain dependent. No fluorescence occurs in King's B medium. Tween 20 and 80 are hydrolyzed. Urease, catalase, oxidase, and lecithinase activities are all positive. Nitrate is reduced, but not nitrite. Gelatin liquefaction is negative. No hydrolysis of casein, starch, or esculin occurs. Arginine deiminase is negative. PHB is accumulated and, according to the description, is utilized. The report does not specifically state whether this refers to extracellular PHB.

The strains assigned to this species belonged to one of the genomovars (genomovar II) into which was subdivided a collection of *B. cepacia* isolated from cases of cystic fibrosis. Four strains of this genomovar shared high DNA sequence similarity as determined by hybridization methods, and the melting temperatures of rRNA–DNA hybrid molecules using *B. cepacia* as reference were lower than the homologous *B. cepacia* reassociated molecules. The phenotypic differences between *B. multivorans* and *B. cepacia* are limited to casein digestion, growth at 42°C, and acid production from sucrose and raffinose. In fact, these last two properties may be redundant, since the two saccharides may be hydrolyzed by the same enzyme, for instance, invertase. Acid may be produced from the resulting monosaccharides (glucose and fructose). Since the physiological properties used for the description of this species differ from those used for other species of the genus, it is not possible to use them in a more extensive comparison, and therefore the species has been excluded from the comparative Table BXII.β.3.

The mol% G + C of the DNA is: 68–69 (method unknown).

Type strain: LMG 13010, ATCC BAA-247, CCUG 34080, CIP 105495, DSM 13243, NCTC 13007.

GenBank accession number (16S rRNA): AF14855.

13. **Burkholderia phenazinium** (Bell and Turner 1973) Viallard, Poirier, Cournoyer, Haurat, Wiebkin, Ophel-Keller and Balandreau 1998, 5618^VP (*Pseudomonas phenazinium* Bell and Turner 1973, 753.)

phe.na.zi′ni.um. Orthography and etymology uncertain; possibly refers to iodinin, which is a phenazine pigment.

The original bacteriological information on this species is fragmentary. It is included in a paper describing the production of iodinin under various conditions (Bell and Turner, 1973). The strain was isolated from soil after an enrichment using L-threonine as the sole carbon source. This property is mentioned by Viallard et al. (1998) in their short description. It has no value whatsoever as a diagnostic character, since many aerobic pseudomonads use this amino acid and the property is usually shared by all the strains of a given species.

An unusual feature mentioned in the original description of the species is its acidophilic character. The strain was acidophilic, the optimal pH for growth being 5.0. It failed to grow at pH 7.0. This feature apparently has not been mentioned by later researchers who have worked with this strain. Colonies on agar media produced iodine crystals, and the presence of L-threonine or glycine in the medium seemed to enhance pigment synthesis. In experiments designed to test iodinin production, growth was observed with threonine, glycine, fumarate, glycerol, sucrose, malate, succinate, DL-lactate, citrate, glucose, glutamate, pyruvate, and serine. Growth was poor with aminopropanol, and no growth was observed with propionate. Additional information, taken from Viallard et al. (1998), is summarized in Table BXII.β.2. The description does not mention specifically use of extracellular PHB as a growth substrate. Acid production from various sugars is given in the original paper.

The mol% G + C of the DNA is: (HPLC).

Type strain: ATCC 33666, DSM 10684, LMG 2247, NCIB 11027.

GenBank accession number (16S rRNA): U96936.

As a general comment, the phenotypic properties of the description for *B. phenazinium* do not clearly differentiate this species from *B. cepacia*, and no data on DNA similarity expressed by percent DNA hybridization are reported. Only the $T_{m(e)}$ values of the DNA–rRNA hybrids obtained with 23S rRNA seem to differentiate this species from *B. cepacia*. For the moment, it is advisable to consider this taxon as a genomovar of *B. cepacia* until more evidence is available to decide on its independent species rank.

14. **Burkholderia plantarii** (Azegami, Nishiyama, Watanabe, Kadota, Ochuchi and Fukazawa 1987) Urakami, Ito-Yoshida, Araki, Kijima, Suzuki and Komagata 1994, 242^VP (*Pseudomonas plantarii* Azegami, Nishiyama, Watanabe, Kadota, Ochuchi and Fukazawa 1987, 151.)

plan′tar.i.i. L. n. *plantarium* seedbed; L. gen. n. *plantarii* of seedbed.

The following description is summarized from that in the original paper by Azegami et al. (1987). Nonencapsulated straight rods (0.7–1.0 × 1.4–1.9 µm), motile with one to three polar flagella. They occur singly, in pairs, or in short chains. Colonies have a slightly yellow tint, and they produce a water-soluble reddish brown pigment depending on the conditions and the medium. Oxidase positive. Do not produce fluorescent pigment. Gelatinase, lecithinase, hydrolysis of Tween 80, and denitrification are all positive. Arginine dihydrolase negative. Acid production on a number of carbon compounds and nutritional properties are described in the original paper. Further details are summarized in Tables BXII.β.2, BXII.β.3, and BXII.β.4. The species causes rice seedling blight, and strains have been isolated from rice seedlings and from bed soil in nursery boxes in Japan.

The mol% G + C of the DNA is: 64.8 (Bd).

Type strain: ATCC 43733, AZ 6201, DSM 9509, JCM 5492, LMG 9035, NIAES 1723.

GenBank accession number (16S rRNA): U96933.

15. **Burkholderia pseudomallei** (Whitmore 1913) Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398^VP (Effective publication: Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1992, 1273) (*Pseudomonas pseudomallei* (Whitmore 1913) Haynes and Burkholder 1957, 100; *Bacillus pseudomallei* Whitmore 1913, 9.)

pseu.do.mal′ le.i. Gr. adj. *pseudes* false; L. n. *malleus* the disease glanders; M.L. gen. n. *pseudomallei* of false glanders.

The general characteristics of the species and properties useful for differentiation from the related species *B. mallei* are given in Tables BXII.β.2, BXII.β.3, and BXII.β.7. Colonies can range in structure from extremely rough to mucoid, and in color from cream to bright orange. Optimal growth temperature, ~37°C. For further descriptive information see Redfearn et al. (1966) and Redfearn and Palleroni (1975). Isolated from human and animal cases of melioidosis and from soil and water in tropical regions, particularly Southeast Asia. Probably a soil organism and accidental pathogen, causing melioidosis.

The mol% G + C of the DNA is: 69.5 (Bd).

Type strain: ATCC 23343, NCTC 12939, WRAIR 286.

16. **Burkholderia pyrrocinia** (Imanaka, Kousaka, Tamura and Arima 1965) Vandamme, Holmes, Vancanneyt, Coenye, Hoste, Coopman, Revets, Lauwers, Gillis, Kersters and Govan 1997b, 1199[VP] (*Pseudomonas pyrrocinia* Imanaka, Kousaka, Tamura and Arima 1965, 205.)

pyr.ro.ci′ni.a. Etymology uncertain, possibly M.L. adj. *"pyrrocin"* referring to the antibiotic properties of pyrrolnitrin, which is produced by strains of this species.

The following description is taken from the original paper. Rods, 0.5–0.8 × 1.2–2.0 μm, occurring singly. Motile by means of polar flagella. No pigment is produced. Oxidase reaction, nitrate reduction, denitrification, and starch hydrolysis are all negative. H₂S is produced. Optimal temperature for growth, 26–30°C. Growth is scanty at 37°C and negative at 42°C. Acid produced from glucose, galactose, lactose, sucrose, and glycerol, but not from maltose, trehalose, mannose, raffinose, starch, or inulin. 2-Ketogluconate is produced from gluconate.

Growth on glucose, gluconate, 2-ketogluconate, and *p*-hydroxybenzoate as sole carbon sources. 5-Ketogluconate, citrate, ethanol, phenol, succinate, benzoate, salicylate, *m*-hydroxybenzoate, protocatechuate, gentisate, anthranilate, and *p*-aminobenzoate are not utilized for growth. Some of the properties are summarized in Table BXII.β.2. The strains produce the antibiotic pyrrolnitrin. This compound is also produced by some strains of *Pseudomonas chlororaphis*, *P. aureofaciens*, and *Burkholderia cepacia* (*"Pseudomonas multivorans"*) (Elander et al., 1968), indicating that the synthesis occurs in organisms of different branches of the *Proteobacteria*.

The first suggestion of allocation of *B. pyrrocinia* to the RNA similarity group II was made by Byng et al. (1980). In their proposal for the transfer of *Pseudomonas pyrrocinia* to the genus *Burkholderia*, Vandamme et al. (1997b) add to the above description, the characteristic fatty acid composition. Allocation to the genus *Burkholderia* also has been recommended by Viallard et al. (1998) based on nucleic acid sequencing studies.

The mol% G + C of the DNA is: 65.

Type strain: ATCC 15958, DSM 10685, LMG 14191.

GenBank accession number (16S rRNA): U96930.

17. **Burkholderia thailandensis** Brett, Deshazer and Woods 1998, 318[VP]

thai.lan.den′ sis. M.L. adj. *thailandensis* pertaining to Thailand, where the organism was originally isolated.

Rods, motile due to the presence of two to four polar flagella. Colonies are smooth and glossy with a pink pigmentation on modified Ashdown's selective medium (see Enrichment and Isolation Procedures), while *B. pseudomallei* colonies are rough and wrinkled, with a dark purple pigmentation. The API 20NE and API 50CH biochemical profiles are similar to those of *B. pseudomallei*. Exceptions are the capacity to use L-arabinose, 5-ketogluconate, and adonitol, and the inability to utilize erythritol and dulcitol. Strains of the species are avirulent for Syrian golden hamsters. Growth occurs at temperatures between 25°C and 42°C. Production of siderophore, lipase, lecithinase, and protease are positive. The strains are resistant to aminoglycosides but sensitive to tetracycline, ceftazidime, and trimethoprim. Type strain was isolated from a rice field soil sample in central Thailand.

The mol% G + C of the DNA is: unknown.

Type strain: E264, ATCC 700388.

GenBank accession number (16S rRNA): U91838.

A comparison of sequences of 16S rRNA genes indicates a close relationship of *B. thailandensis* to *B. pseudomallei*. No DNA–DNA hybridization experiments between strains of the two species have been reported. In view of the low resolving power of 16S rRNA sequence similarity at the species level and the limited number of phenotypic differences, it is hoped that future studies may provide evidence supporting the taxonomic position of this group of organisms as an independent species of *Burkholderia*.

18. **Burkholderia vandii** Urakami, Ito-Yoshida, Araki, Kijima, Suzuki and Komagata 1994, 242[VP]

van′ di.i. M.L. gen. n. *vandii* of *Vanda*, a genus of orchids.

Description taken from Urakami et al. (1994). The cells are 0.5–1.0 × 1.5–3.0 μm and have rounded ends. They occur singly, rarely in pairs, and are motile by one or several polar flagella. Abundant growth occurs in nutrient broth and peptone water. The colonies are white to light yellow. No diffusible fluorescent pigment is produced.

The methyl red and the Voges–Proskauer reactions are negative. Indole and hydrogen sulfide are not produced. Starch is not hydrolyzed. Denitrification and hydrolysis of gelatin are positive. Acids are weakly produced from inositol and glycerol oxidatively, but not from L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, or soluble starch. No fermentation of sugars occurs. Nitrate is not used as a nitrogen source. Results of nutritional studies at the expense of many organic compounds, as well as extensive physiological information, are given in the original paper (Urakami et al., 1994) and in Gillis et al. (1995). The single known strain was isolated from orchids of the genus *Vanda* as an antibiotic-producing bacterium active against the plant pathogenic organisms *Clavibacter michiganensis* and *Fusarium oxysporum*.

According to Coenye et al. (1999b), *B. vandii* is a junior synonym of *B. plantarii*.

The mol% G + C of the DNA is: 68.5 (HPLC).

Type strain: ATCC 51545, DSM 9510, JCM 7957, LMG 16020, VA-1316.

GenBank accession number (16S rRNA): U96932.

Studies by Coenye et al. (1999b) based on SDS-PAGE of whole cell proteins, DNA–DNA hybridization, and extensive

biochemical characterization indicate that *B. vandii* should be considered a junior synonym of *B. plantarii*.

19. **Burkholderia vietnamiensis** Gillis, Van, Bardin, Goor, Hebbar, Willems, Segers, Kersters, Heulin and Fernandez 1995, 287[VP]

vi.et′ na.mi.en.sis. M.L. adj. *vietnamiensis* referring to Vietnam, where the rice strains were isolated.

Motile cells are 0.8–2 × 0.3–0.8 µm. No details on the number of flagella per cell are given. Colonies on nutrient agar are not pigmented and do not produce fluorescent pigment on King B medium, a property that is uniformly negative for species of *Burkholderia*. Growth occurs on nutrient agar between 20°C and 41°C. Strains are oxidase, catalase, β-galactosidase, and gelatinase positive. All strains fix N_2 and produce ornibactin siderophores, but not pyochelin or cepabactin. Nutritional properties and characteristics useful for differentiation from other *Burkholderia* species have been summarized in Tables BXII.β.2 and BXII.β.3.

Further details are to be found in Gillis et al. (1995). In one of the tables in that article, denitrification is recorded as positive, although the property is not specifically mentioned in the text, where *B. vietnamiensis* is described as capable of reducing nitrate to nitrite. Further reduction to gases (N_2O or N_2) and the ability to grow under anaerobic conditions in the presence of nitrate are not mentioned. It may be inferred that strains of this species, as those of *B. cepacia*, are unable to denitrify. Differences between *B. vietnamiensis* and *B. cepacia* are in the utilization of L-arabitol, adonitol, butylamine, tryptamine, citraconate, and 5-ketogluconate by *B. cepacia*, and the ability of *B. vietnamiensis* to grow on itaconate. Strains of the latter do not synthesize the siderophores cepabactin and pyochelin (Gillis et al., 1995). *B. vietnamiensis* is capable of N_2 fixation, a property recorded earlier for rhizosphere strains of *B. cepacia* (Bevivino et al., 1994). However, extracts from clinical strains also gave a single hybridization signal with a *nifA* probe from *Klebsiella pneumoniae*, although they did not fix nitrogen (Tabacchioni et al., 1995). Interestingly, the clinical strains produced pyochelin and its precursor, salicylic acid, a property absent from the rhizosphere strains (Bevivino et al., 1994). Therefore, it is very likely that the latter may have corresponded to the taxon to be described later as *B. vietnamiensis* (Gillis et al., 1995).

There is little doubt, however, that *B. cepacia* and *B. vietnamiensis* are closely related, as suggested by their relative position in the tree of Fig. BXII.β.2. Aside from many phenotypic characteristics, their similarity extends to the production of the siderophore ornibactin (Meyer et al., 1995), and to the structure of the putative O-specific polymer isolated from the LPS of *B. vietnamiensis*, which resembles the O-antigen of *B. cepacia* serogroup J (Gaur and Wilkinson, 1996). The strains of *B. vietnamiensis* have been isolated from rice field soils and from clinical specimens. They are not pathogenic for onions.

The mol% G + C of the DNA is: 67.9 (T_m).

Type strain: DSM 11319, LMG 10929, TVV75.

GenBank accession number (16S rRNA): U96928, U96929.

Genus II. **Cupriavidus** *Makkar and Casida 1987a, 325*[VP]

DAVID L. BALKWILL

Cup.ri.a.vi′ dus. L. n. *cuprum* copper; L. adj. *avidus* eager for, loving; M.L. neut. n. *Cupriavidus* lover of copper.

Coccoid rods, 0.7–0.9 × 0.9–1.3 µm. Gram negative. **Motile by two to ten peritrichous flagella. Chemoheterotrophic.** An organic nitrogen source is not required. Glucose not utilized. **Strictly respiratory metabolism** with oxygen as the terminal electron acceptor. Oxidase positive. Catalase positive. Nitrate reduced. Gelatin, starch, and urea not hydrolyzed. Indole and H_2S not produced. **Can use any of several amino acids—but not L-lysine or L-methionine—as the sole source of carbon and nitrogen.** Optimal temperature, 27°C. Optimal pH, 7.0–8.0. NaCl at 3% inhibits growth. **Resistant to copper** at concentrations up to at least 800 µM. Growth initiation is stimulated by copper. Colonies on nutrient agar after 2 d at 27°C are off-white, glistening, mucoid, smooth, and convex, with an entire edge; 2–4 mm in diameter. Isolated from soil. **Nonobligate predator causing lysis of various Gram-positive and Gram-negative bacteria in soil.** Can lyse certain other nonobligate bacterial predators. Growth does not require presence of prey species.

The mol% G + C of the DNA is: 57 ± 1 (T_m).

Type species: **Cupriavidus necator** Makkar and Casida 1987a, 325.

FURTHER DESCRIPTIVE INFORMATION

C. necator cells are Gram-negative short rods measuring 0.7–0.9 × 0.9–1.3 µm, based on electron micrographs of negatively stained cells. The cells decrease somewhat in size and become more rounded as cultures age, or when the organism is placed in contact with soil during soil column studies (Byrd et al., 1985). In soil, the rounded forms of *C. necator* appear to be dormant (Byrd et al., 1985). *C. necator* reproduces by binary fission and is motile by 2–10 peritrichous flagella.

Colonies of *C. necator* on nutrient agar after 2 d of incubation at 27°C are 2–4 mm in diameter, off-white, glistening, mucoid, smooth, and convex, with an entire edge. Nonmucoid variants of *C. necator* that form slightly smaller colonies appear after many transfers on laboratory media. The colonies of these variants are flat and do not glisten. A phage that lyses the mucoid form of the organism does not lyse the nonmucoid form, but the two variants are similar in all other respects.

C. necator grows aerobically on nutrient agar or synthetic medium with L-glutamic acid, but only scant growth occurs on these media under anaerobic conditions. In thioglycolate broth, the organism grows only in the oxidized zone. Good growth occurs in acetate or fructose broth media with NH_4Cl as the sole source of nitrogen. Excellent growth is obtained in N-1 synthetic medium (Makkar and Casida, 1987a) broth with L-glutamic acid as the nitrogen and carbon source. The optimal growth temperature for *C. necator* is 27°C, but it can grow well at 37°C. Growth occurs after a delay at 15°C, but there is no growth at 55°C. *C.*

necator grows over a pH range of 5.5–9.2, and its optimal growth pH is 7.0–8.0. It grows well in media that contain 1% NaCl, but growth is poor at 2% NaCl and inhibited fully at 3% NaCl.

C. necator does not grow in a modified Burk's N-free medium (see Makkar and Casida, 1987a) with glucose or sucrose as the sole source of carbon. Limited growth takes place with fructose but is not sustained during repeated transfers in N-free medium. *C. necator* can grow in a synthetic medium (Makkar and Casida, 1987a) without added magnesium. It is quite resistant to copper and grows well in the presence of 1200 µM CaCl$_2$·2H$_2$O (Casida, 1987). Copper also stimulates growth initiation of *C. necator* but has no significant effect on its subsequent growth rate at concentrations up to at least 800 µM (Casida, 1987; Makkar and Casida, 1987a).

The metabolism of *C. necator* is oxidative, as determined by the Hugh-Leifson test (Hugh and Leifson, 1953) performed in a synthetic medium with fructose as the carbon source. It grows and produces acid without gas in the top portion of aerobic tubes, but does not grow or produce acid in anaerobic tubes (Makkar and Casida, 1987a). *C. necator* is catalase positive and oxidase positive. It does not show hemolysis on blood agar. Hippurate, tributyrin, Tween 20, and Tween 80 are hydrolyzed, but esculin, gelatin, starch, and urea are not hydrolyzed. DNase activity is negative. *C. necator* degrades tyrosine. It does not produce indole or H$_2$S. In litmus milk, the reaction is basic with reduction of the litmus. Acetate, L-aspartate, citrate, fructose, fumarate, gluconate, L-glutamate, glutarate, β-hydroxybutyrate, lactate, L-leucine, oxalacetate, and succinate are utilized as sources of carbon for growth. Use of sucrose as a carbon source is equivocal. Utilization of acetate, fructose, L-glutamate, and L-leucine occurs after a brief delay (13–15 h). Somewhat longer delays are seen with L-alanine, and L-valine (61 and 92 h, respectively; Makkar and Casida, 1987a). *C. necator* does not utilize arabinose, adonitol, benzoate, glucose, glycerol, lactose, L-lysine, mannitol, mannose, melibiose, L-methionine, rhamnose, or xylose as carbon sources for growth.

The mol% G + C content of *C. necator* chromosomal DNA is 57 ± 1, as determined by the thermal melting point technique (Makkar and Casida, 1987a). The 16S rRNA gene sequence for the type strain of *C. necator* (ATCC 43291) has been determined (Balkwill, unpublished data; GenBank accession no. AF191737). Phylogenetic analyses of this sequence with distance matrix (see Fig. BXII.β.3), parsimony, and maximum likelihood methods indicated that *C. necator* should be assigned to the *Burkholderiaceae*. Moreover, all of the analytical methods placed *C. necator* within a cluster of strains representing the genus *Ralstonia* (Yabuuchi et al., 1995). *C. necator* was most closely related to two strains (including the type strain) of *Ralstonia eutropha* that were included in the analysis. Its 16S sequence was virtually identical to those of the two *Ralstonia* strains, differing from them by only a single base over 1338 bases compared (sequence similarity 99.9%). Technically, this high sequence similarity alone does not demonstrate that *C. necator* and *R. eutropha* are the same species. Stackebrandt and Goebel (1994) have suggested that DNA–DNA reassociation values should be used to determine whether strains of bacteria are members of the same species when their 16S rDNA sequence similarities are greater than 97.5%. Nevertheless, the phylogenetic analyses do provide strong evidence that *C. necator* is a member of the genus *Ralstonia*.

The habitat for *Cupriavidus* is soil. *C. necator* is a nonobligate predator that can cause lysis of various types of Gram-positive and Gram-negative bacteria in soils, including several other non-

obligate predator species. However, growth of *C. necator* in soil or laboratory media does not require the presence of prey species. *C. necator* produces the heat-labile, magnesium-related growth initiation factor (GIF) that was first described by Casida (1984) and that is produced by various types of bacteria in soils. This GIF can initiate the growth of *Agromyces ramosus* and other species in soil (Casida, 1984; Byrd et al., 1985), thereby increasing the supply of suitable prey cells for nonobligate bacterial predators like *C. necator*. The actual mechanism by which *C. necator* kills its prey cells is not yet known, although Casida (1987) has suggested that a copper-related peptide used to scavenge copper from the environment (see below) might also be used to deliver excess (and toxic) amounts of copper to prey cells.

Zeph and Casida (1986) used an indirect phage analysis method involving soil percolator columns and plaque assays (see Byrd et al., 1985) to assess the ability of *C. necator* and several other nonobligate bacterial predators to attack various types of potential prey species in soil. Of the 11 predators examined, *C. necator* strain N-1 (the type strain) and an uncharacterized predator strain (designated L-2) were capable of attacking the widest variety of prey species. Nonpredatory bacteria attacked by *C. necator* included *Arthrobacter globiformis*, *Azotobacter vinelandii*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Escherichia coli*, *Micrococcus luteus*, and *Staphylococcus aureus*. Other nonobligate bacterial predators attacked by *C. necator* included *Agromyces ramosus*, *Ensifer adhaerens*, strain C2 (an uncharacterized predator), and a streptomycete-like isolate (strain 3). *C. necator* did not attack *Agrobacterium tumefaciens*, *Nocardia salmonicolor*, *Salmonella typhi* (all of which are nonpredatory), or four uncharacterized predatory strains, including L-2. *C. necator* itself was attacked by *A. ramosus* and one uncharacterized predatory strain, but was not attacked by *E. adhaerens* or seven other uncharacterized predators. Comparison of prey ranges for different predators indicated that Gram-negative predators like *C. necator* are likely to be more important than Gram-positive predators, all of which had comparatively narrow prey ranges. It is also possible that nonobligatory predators such as *C. necator* control the populations of Gram-positive predators (like *A. ramosus*), and thus might be at the top of the hierarchy of bacterial predators in soils.

When *C. necator* interacts with *Agromyces ramosus* in a soil, a predator-on-predator attack and counterattack phenomenon occurs (Byrd et al., 1985). *A. ramosus* is a Gram-positive species that occurs in high numbers in soils and functions as a nonobligatory predator that destroys cells of several other bacterial species. It typically occurs in soils as coccoid rods but can produce a mycelium that eventually fragments. Within 2 d after *A. ramosus* and *C. necator* are added to a soil, *A. ramosus* forms a mycelium wherever the *C. necator* cells are situated, and roughly one-third of the *C. necator* cells become ghosted. *C. necator* apparently produces the magnesium-related GIF cited above, which stimulates the growth of *A. ramosus* and subsequent development of its mycelium. While this occurs, however, *A. ramosus* attacks and lyses some of the *C. necator* cells. The mycelium appears to deliberately seek out the cells of *C. necator*, possibly to make contact with them. Lysis of the *A. ramosus* mycelium in contact with cells of *C. necator* is seen within 4–5 d after adding the two bacteria to a soil. Proliferation of *C. necator* wherever the *A. ramosus* mycelium grew is also seen by this time, indicating that *C. necator* has counterattacked *A. ramosus*. *C. necator* only attacks the mycelial form of *A. ramosus*, so portions of the mycelium that fragment to the rod form before *C. necator* can attack them survive the attack-
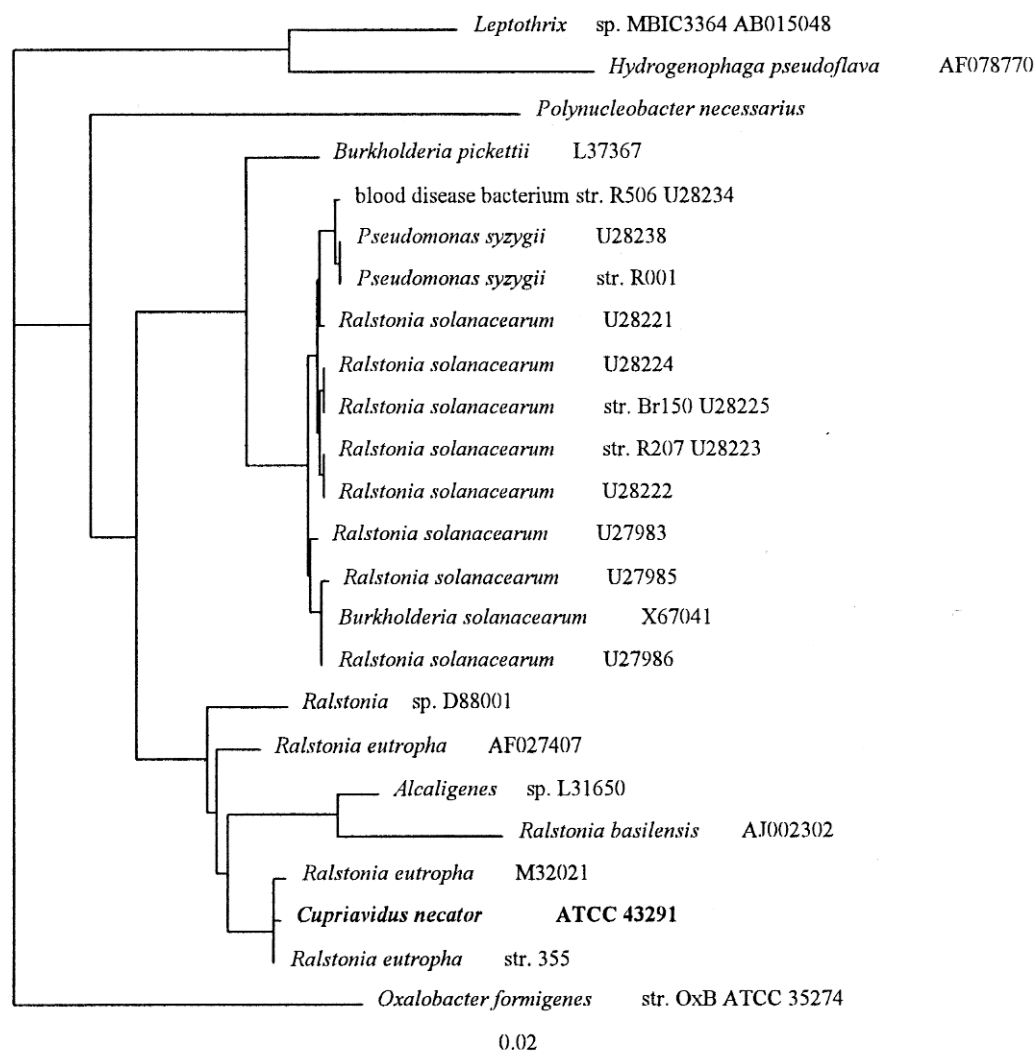
FIGURE BXII.β.3. Phylogenetic tree for *Cupriavidus necator* ATCC 43291 and selected strains of eubacteria, based on a distance matrix analysis. Bar = 2 substitutions per 100 bases. *Oxalobacter formigenes* was used as the outgroup. Parsimony and maximum likelihood analyses yielded virtually identical trees with respect to the positions of *C. necator* and *Ralstonia eutropha* and the branching patterns within the cluster containing strains of these two organisms. The numbers in parentheses are the GenBank/ EMBL accession numbers for the sequences used in the analysis. The accession number for the *C. necator* 16S rDNA sequence is AF191737.

counterattack interaction. This might explain why *A. ramosus* maintains high numbers in soils despite being a prey organism.

Casida (1989a) investigated the possibility that *Arthrobacter globiformis*, which is usually present in relatively high numbers in soils, might serve as a major reservoir of prey cells for *C. necator* and other copper-resistant Gram-negative bacterial predators. This study found that a large increase in the culturable numbers of an uncharacterized bacterium that hydrolyzes GELRITE occurs within 2.5 h. Because of the short time period involved, it was felt that exposure to *A. globiformis* caused the hydrolyzers to break dormancy (and thus become more readily culturable), rather than to multiply extensively. The numbers of *C. necator* and other Gram-negative predator cells increased in response to addition of *A. globiformis* cells as well. However, as the numbers of predator cells increased, the levels of hydrolyzers in the soil decreased extensively while the numbers of *A. globiformis* cells did not. It appeared, then, that the predators were responding directly to the hydrolyzers that were activated after *A. globiformis*

was added to the soil, rather than to *A. globiformis* itself. If so, *A. globiformis* most likely does not serve as the major reservoir of prey cells for *C. necator* and other copper-resistant Gram-negative predators, but it may play a role in enabling them to access prey cells such as the hydrolyzers.

Casida (1989b) also investigated the possibility that protozoa might be major predators of *C. necator* and other bacterial predators in soil. Protozoa that can attack *C. necator* were detected in soil, but no evidence was found that would indicate these protozoa and *C. necator* actually interact with each other in soils.

As noted above, *C. necator* is resistant to copper, and copper stimulates initiation of its growth. Casida (1987) found that growth initiation is delayed 13–120 h when *C. necator* is transferred to a synthetic medium with various carbon sources, the actual time depending on which carbon source is supplied in the medium. During this delay period, *C. necator* produces a GIF that is different from the heat-labile magnesium-associated GIF that it uses to stimulate the growth of *A. ramosus* and other bac-

teria in soil (Byrd et al., 1985). The newly discovered GIF was shown to specifically stimulate growth initiation of *C. necator* and to be a copper-related, heat-stable peptide with a molecular weight greater than 10,000 and a relatively low cysteine content. It is thought that *C. necator* uses this heat-stabile peptide GIF to marshal copper from the surrounding environment, as elevated levels of copper are apparently needed to initiate growth. After growth has been initiated, however, copper has no significant effect on the growth rate and production of the copper-associated peptide GIF stops. Casida (1987) also found that the copper-associated GIF strongly inhibits the growth of *A. ramosus*, perhaps by delivering lethal levels of copper to its cells. If so, *C. necator* may use a twofold strategy when it interacts with *A. ramosus* in soil. First, it could produce the heat-labile magnesium-associated GIF to stimulate growth of *A. ramosus* and development of the mycelium. Then, it could use the heat-stable copper-associated GIF to kill the mycelial cells.

ENRICHMENT AND ISOLATION PROCEDURES

C. necator can be enriched and isolated from soils with a baiting technique developed by Sillman and Casida (1986). This method appears to utilize chemotaxis, although that has not been confirmed. Air-dried soil is passed through a fine sieve and placed to a depth of 3–4 mm in a sterile Petri plate. This soil is tamped lightly to produce a uniformly flat surface, and about 2 ml of sterile distilled water is added along the walls of the plate. The soil should be moist but not flooded. A 0.65-μm pore size membrane filter is next placed on a circle (8.5 mm diameter) of sterile Whatman no. 2 filter paper in a separate sterile Petri plate. A concentrated washed suspension of early stationary-phase host cells (of *Micrococcus luteus*) is spread over a 28 mm diameter area at the center of the membrane filter, and excess moisture is absorbed by the underlying filter paper. The membrane filter is then placed on the surface of the soil with the side containing the host cells facing upward. After gently pressing the edges of the filter against the soil, the Petri plate is sealed with tape and incubated (right side up) at 27°C. Several filters are prepared in this way, so that one can check for the appearance of predators daily as incubation continues.

The following procedure is used to check for the appearance of predators during incubation. The host cells are removed from a filter by gentle scraping with an alcohol-flamed spatula and suspended in 5 ml of sterile tap water. This suspension is then mixed and diluted through a 1:2 dilution series. Aliquots (0.1 ml) of each dilution and the undiluted suspension are placed on the surfaces of agar plates, along with 0.1 ml of a fresh, concentrated host-cell suspension, and aseptically spread over the surfaces of the plates. Either 1.5% Noble agar (in distilled water) or a very dilute nutrient medium (such as 0.01 strength heart infusion broth) can be used in the plates for this part of the isolation procedure. The plates are then incubated for at least 3 d and checked for the development of plaques, colonies, or colonies within plaques. These are picked with a needle and spread through freshly prepared lawns of host cells on new plates of the same medium. After a sufficient period of incubation at 27°C, isolates growing on the plates are restreaked for purification on 0.01 strength heart infusion agar without added host cells. This approach establishes that the isolates are not obligatory predators.

C. necator typically appears on the initial isolation filters within 3 d of incubation at 27°C. Isolates can be tested for their ability to attach to host cells as follows. Microscope slides are positioned vertically along the inside walls of 250-ml wide-mouth Erlenmeyer flasks. The slides are then held in place by adding sterile glass beads to a depth of 4 cm. Sufficient sterile tap water is added to nearly submerge the glass beads, and host cells of *M. luteus* are added to produce a turbid suspension. One loopful of potential predator cells is also added, and the flasks are incubated on a rotary shaker at 27°C. After incubation, the slides are removed from the flask, stained with crystal violet, and examined for predator attachment to host. This should be done both at the splash line and on the part of the slide that was submerged.

The above procedure has been used to isolate a number of Gram-negative, nonobligate bacterial predators from soil, including several copper-resistant strains. If one in interested in isolating *C. necator* specifically, then it will be necessary to screen isolates for the key descriptive characteristics of this species.

MAINTENANCE PROCEDURES

C. necator can be maintained on 0.1-strength heart infusion agar. It can be lyophilized by common procedures used for aerobes. It can also be preserved by freezing in cryoprotectant and subsequent storage at −80°C or under liquid nitrogen.

DIFFERENTIATION OF THE GENUS *CUPRIAVIDUS* FROM OTHER GENERA

When the genus *Cupriavidus* was proposed by Makkar and Casida in 1987a, it was most similar to *Alcaligenes*. As a result it was compared directly to that genus, especially to *Alcaligenes faecalis*. Among the characteristics that both organisms had in common were cell morphology, the mol% G + C content of their DNA, and lack of the ability to utilize glucose as a sole carbon source. However, *C. necator* could be differentiated from *A. faecalis* on the basis of five traits: lack of a requirement for added magnesium in synthetic media, its predatory activity, ability to utilize fructose as a carbon source, copper resistance, and stimulation of growth initiation by elevated levels of copper. In all likelihood, the combination of copper resistance and predatory behavior also differentiates *Cupriavidus* from other Gram-negative aerobic rods.

Analyses of 16S rRNA gene sequences indicate that *C. necator* is a member of the *Burkholderiaceae* and is phylogenetically situated within the genus *Ralstonia* (Yabuuchi et al., 1995). Among comparable phenotypic traits published to date, *C. necator* differs from all currently recognized species of *Ralstonia* only in that it cannot utilize glycerol as a carbon source (see Makkar and Casida, 1987a; Yabuuchi et al., 1992, 1995; Urakami et al., 1994; Gillis et al., 1995; Coenye et al., 1999a; Vandamme et al., 1999). However, *C. necator* differs from the phylogenetically most closely related species of *Ralstonia* (*R. eutropha*) in two other ways: it does not utilize benzoate as a carbon source and it does not cause hemolysis on blood agar. *C. necator* also differs from all recognized *Ralstonia* species in that it has a considerably lower mol% G + C content (57 ± 1 versus 64–68.3).

TAXONOMIC COMMENTS

Phylogenetic analysis data and the apparent paucity of phenotypic differences between *C. necator* and *Ralstonia* spp. raise serious doubts that *Cupriavidus* and *Ralstonia* are distinct genera. The predatory behavior of *Cupriavidus* might distinguish it from *Ralstonia*, but no *Ralstonia* species have been tested for this characteristic. The reported mol% G + C content for *C. necator* (57 ± 1) is outside the range of values reported for *Ralstonia* spp. (64–68.3), and all determinations were done by the same

method. A thorough direct comparison of *C. necator* with all recognized *Ralstonia* spp. might detect additional differences. Based on information available at this time, however, it appears that *C. necator* should be transferred to the genus *Ralstonia*.

Phylogenetic analyses indicated that *C. necator* and *Ralstonia eutropha* have virtually identical 16S rRNA gene sequences. At noted earlier, this very high sequence similarity does not prove that *C. necator* and *R. eutropha* are the same species; DNA–DNA reassociation values must be determined to resolve that question (see Stackebrandt and Goebel, 1994). This has not been done for *C. necator* and *R. eutropha*, but their differing mol% G + C contents (see above) imply that the reassociation values resulting from such an experiment might be quite low. Moreover, several phenotypic differences between these two strains have been reported in the literature (see above). Copper resistance and predatory behavior could be additional differences, but *R. eutropha*

has not been examined for these traits. In addition, the copper resistance in *Cupriavidus* could be plasmid-coded (as metal resistance often is) and, if so, it should not be considered a characteristic that defines the species. At this point, there is insufficient information to know whether *C. necator* and *R. eutropha* are distinct species. DNA–DNA hybridization studies would be most helpful in clarifying the situation.

List of species of the genus Cupriavidus

1. **Cupriavidus necator** Makkar and Casida 1987a, 325[VP]

ne.ca′ tor. L. n. *necator* slayer.

The characteristics are as described for the genus. During growth under laboratory conditions, *C. necator* may produce small numbers of a nonmucoid variant. The variant resembles the mucoid form except that its colonies are

smaller and display a drier, flatter appearance. However, a bacteriophage isolated from soil that lyses the mucoid form does not lyse the nonmucoid variant.

The habitat is soil.

The mol% G + C of the DNA is: 57 ± 1 (T_m).

Type strain: ATCC 43291.

GenBank accession number (16S rRNA): AF191737.

Genus III. **Lautropia** Gerner-Smidt, Keiser-Nielsen, Dorsch, Stackebrandt, Ursing, Blom, Christensen, Christensen, Frederiksen, Hoffmann, Holten-Andersen and Ying 1995, 418[VP] (Effective publication: Gerner-Smidt, Keiser-Nielsen, Dorsch, Stackebrandt, Ursing, Blom, Christensen, Christensen, Frederiksen, Hoffmann, Holten-Andersen and Ying 1994, 1795)

PETER GERNER-SMIDT

Lau.tro′ pi.a. M.L. fem. n. *Lautropia* of Lautrop, named after Hans Lautrop.

Cocci may occur in at least **three forms: (1) encapsulated**, nonmotile 1–2 μm in diameter, forming aggregates of 10 to >100 cells; **(2) unencapsulated**, nonaggregated 1–2 μm in diameter, **motile** by a tuft of three to nine flagella; and **(3)** large (>5 μm in diameter), **spherical nonaggregated, nonmotile**. Do not form endospores. **Gram negative.** Nonpigmented. **Grow only on enriched media. Facultative**, but grow best under aerobic conditions. CO_2 is not required. Growth occurs between 30 and 44°C. **Oxidase, catalase, and urease positive.** A polysaccharide is produced on sucrose agar. Various carbohydrates are fermented. Belongs phylogenetically to a separate branch of the class *Betaproteobacteria*. Habitat: the oral cavity and upper respiratory tract of humans.

The mol% G + C of the DNA is: 65.

Type species: **Lautropia mirabilis** Gerner-Smidt, Keiser-Nielsen, Dorsch, Stackebrandt, Ursing, Blom, Christensen, Christensen, Frederiksen, Hoffmann, Holten-Andersen and Ying 1995, 418[VP] (Effective publication: Gerner-Smidt, Keiser-Nielsen, Dorsch, Stackebrandt, Ursing, Blom, Christensen, Christensen, Frederiksen, Hoffmann, Holten-Andersen and Ying 1994, 1795.)

FURTHER DESCRIPTIVE INFORMATION

Rough to smooth colonies grow on many plating media and are composed of extremely pleomorphic cocci with round cells with diameters from 1 to >10 μm. The smallest cells are often motile with circular movements. Glucose, fructose, sucrose, and mannitol are fermented.

An aggregate-forming coccus identified as *L. mirabilis* was the predominant microorganism in sputa from a cystic fibrosis patient on consecutive days (Ben Dekhil et al., 1997c). *L. mirabilis* has also been isolated from the oral cavities of 32 of 60 children infected with human immunodeficiency virus (HIV) and 3 of 25 HIV-uninfected controls (Rossmann et al., 1998); however, the bacterium was not associated with clinical disease in these children.

ENRICHMENT AND ISOLATION PROCEDURES

The organism may be isolated on most nonselective enriched media after aerobic incubation for 1–2 d at 35°C. Microscopy of wet mounts of different colony types is essential to identify the organism in mixed cultures. No selective enrichment procedures have been described for this organism.

MAINTENANCE PROCEDURES

Lautropia cells can either be stored in 10% glycerol broth at −80°C or lyophilized.

DIFFERENTIATION OF THE GENUS *LAUTROPIA* FROM OTHER GENERA

Organisms of the genus *Lautropia* are differentiated from other organisms by their requirement for enriched media, their characteristic colony morphology, their microscopic appearance, and their positive oxidase, urease, and nitrate reduction reactions.

TAXONOMIC COMMENTS

In 1994, Gerner-Smidt et al. (1994), isolated six strains of an organism that seemed to be identical to an organism that had been described by Ørskov (1930). 16S rRNA sequencing revealed that the organisms belonged to class *Betaproteobacteria* of the phylum *Proteobacteria*, separate from all other described genera, but most closely related to *Burkholderia* (Gerner-Smidt et al., 1994). The organisms were assigned to a new genus, *Lautropia*, as the species *L. mirabilis.*

List of species of the genus Lautropia

1. **Lautropia mirabilis** Gerner-Smidt, Keiser-Nielsen, Dorsch, Stackebrandt, Ursing, Blom, Christensen, Christensen, Frederiksen, Hoffmann, Holten-Andersen and Ying 1995, 418^VP (Effective publication: Gerner-Smidt, Keiser-Nielsen, Dorsch, Stackebrandt, Ursing, Blom, Christensen, Christensen, Frederiksen, Hoffmann, Holten-Andersen and Ying, 1994, 1795.)

mi.ra′ bi.lis. L. n. *mirabilis* wonderful.

The characteristics are as described for the genus and as follows. Grows on most enriched media, especially on chocolate, Levinthal, tryptose glucose yeast (TGY) extract, and Tween 80 agar. At least three colony morphologies are seen: (1) flat, dry, and circular; (2) larger, wrinkled, crisp, and crateriform; and (3) smooth, glistering, raised, round, and mucoid. Colonies adhere to the substrate; diameter of the colonies between pinpoint size and >5 mm. Growth in broth granular with a coarse sediment and granules adherent to the side of the tube. Acid is produced from D-glucose, D-fructose, maltose, sucrose, and mannitol; acid is not produced from lactose, trehalose, raffinose, inulin, salicin, adonitol, dulcitol, sorbitol, inositol, D-xylose, L-rhamnose, and L-arabinose. Negative for lysine decarboxylase, ornithine decarboxylase, arginine decarboxylase/dihydrolase, phenylalanine deaminase, Voges–Proskauer test, gelatinase, starch hydrolysis, and H_2S. Hippurate and Tween 80 not hydrolyzed. Most strains hydrolyze esculin. Some strains produce β-xylosidase. β-Galactosidase and β-glucuronidase are not produced.

Rossmann et al. (1998) found variable reactions for arginine dihydrolase, lysine decarboxylase, Voges–Proskauer test, gelatinase at 35°C, and sorbitol fermentation using a commercial identification system (API20E).

Unlike the strains isolated by Gerner-Smidt et al. (1994), the strain isolated by Ben Dekhil et al. (1997c) was hemolytic on blood agar. Rossmann et al. (1998) reported that, with their strains, the catalase test was weakly positive with 10% hydrogen peroxide, and catalase was undetectable with 3% H_2O_2.

Sensitive to penicillin G, ampicillin, piperacillin, cefuroxime, gentamicin, and erythromycin.

The mol% G + C of the DNA is: 64.6–65.4 (T_m).

Type strain: AB2188, ATCC 51599, CCUG 34794, NCTC 12852.

GenBank accession number (16S rRNA): X73223.

Additional Remarks: The type strain was isolated from human dental plaque.

Genus IV. **Pandoraea** Coenye, Falsen, Hoste, Ohlen, Goris, Govan, Gillis and Vandamme 2000, 895^VP

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Pan.do.rae′ a. N.L. fem. n. *Pandoraea* referring to Pandora's box in Greek mythology, the origin of diseases of mankind.

Gram-negative, **motile**, **nonsporeforming rods** (0.5–0.7 × 1.5–4.0 μm). **One polar flagellum.** Positive reactions: catalase, alkaline phosphatase, leucine arylamidase; assimilation of D-gluconate, L-malate, and phenylacetate; **growth on Drigalsky agar** and in 0.5 and 1.5% NaCl. Major fatty acids include $C_{12:0}$, $C_{12:0\ 2OH}$, $C_{16:0}$, $C_{16:0\ 2OH}$, $C_{16:0\ 3OH}$, $C_{17:0\ cyclo}$, $C_{18:1\ 2OH}$, and $C_{19:0\ cyclo\ \omega 8c}$.

The mol% G + C of the DNA is: 61.2–64.3.

Type species: **Pandoraea apista** Coenye, Falsen, Hoste, Ohlen, Goris, Govan, Gillis and Vandamme 2000, 896.

FURTHER DESCRIPTIVE INFORMATION

Negative reactions include nitrite reduction and denitrification; liquefaction of gelatin; hydrolysis of esculin, poly-β-hydroxybutyrate, and Tween 80; production of acid or sulfide in TSI agar; production of indole; production of N-acetyl-β-glucosaminidase, C_{14}-lipase, chymotrypsin, DNase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, lysine decarboxylase, α-mannosidase, ornithine decarboxylase, trypsin, tryptophanase, and valine arylamidase; growth in 10% lactose and 6% NaCl; and growth in O/F medium containing adonitol, fructose, or xylose.

All strains grow on *Burkholderia cepacia* selective medium. Most strains of *Pandoraea* spp. have been isolated from clinical samples, primarily from respiratory tracts of patients suffering from cystic fibrosis but also from blood and from nonclinical sources such as water, sludge, soil, and dried milk (Coenye et al., 2000; Moore et al., 2001).

Coenye et al. (2000) and Daneshvar et al. (2001) provide tables of phenotypic traits that distinguish the five species and four described genomospecies of the genus *Pandoraea*. Coenye et al. (2001b) have described PCR primers that can be used to distinguish among the species of the genus *Pandoraea*. Coenye and LiPuma (2002) have described a PCR-RFLP scheme based on the *gyrB* locus that distinguishes four of the five *Pandoraea* species from each other and from members of three of the four genomospecies described by Daneshvar et al. (2001).

DIFFERENTIATION OF THE GENUS *PANDORAEA* FROM OTHER GENERA

Because of their phenotypic similarity to *Burkholderia cepacia* and the importance of accurate identification for the treatment of cystic fibrosis patients, considerable effort has been devoted to the development of methods to distinguish *Pandoraea* spp. from *B. cepacia* and other organisms found in the respiratory tracts of

cystic fibrosis patients. Phenotypic tests that distinguish *Pandoraea* species from *B. cepacia* genomovars I, III, and IV; *B. multivorans*; *B. vietnamiensis*; *Ralstonia paucula*; and *R. pickettii* are given by Coenye et al. (2000). Phenotypic tests that distinguish *Pandoraea* species from *B. cepacia* genomovars I–VII, *B. gladioli*, and *R. pickettii* are given by Henry et al. (2001). Coenye et al. (2001b) have described PCR primers that can be used to separate isolates belonging to the genus *Pandoraea* from selected members of the genera *Alcaligenes*, *Burkholderia*, *Pseudomonas*, *Ralstonia*, and *Stentrophomonas* as well as distinguishing among the species of the genus *Pandoraea*.

List of species of the genus Pandoraea

1. **Pandoraea apista** Coenye, Falsen, Hoste, Ohlen, Goris, Govan, Gillis and Vandamme 2000, 896[VP]
   a.pis′ ta. Gr. adj. *apistos* disloyal, unfaithful, treacherous.

   As described for the genus with the following additional characteristics. Does not reduce nitrate. Grows on cetrimide agar; grows at 42°C; does not grow in O/F medium with glucose. Does not grow on acetamide. Assimilates caprate, citrate, and DL-lactate; does not assimilate maltose or sucrose. Produces amylase, arginine dihydrolase, C8-ester lipase, phosphoamidase, and urease.

   The mol% G + C of the DNA is: 61.8 (HPLC).
   Type strain: ATCC BAA-61, CCUG 38412, CIP 106627, LMG 16407.
   GenBank accession number (16S rRNA): AF139173.

2. **Pandoraea norimbergensis** (Wittke, Ludwig, Peiffer and Kleiner 1998) Coenye, Falsen, Hoste, Ohlen, Goris, Govan, Gillis and Vandamme 2000, 896[VP] (*Burkholderia norimbergensis* Wittke, Ludwig, Peiffer and Kleiner 1998, 631)
   no.rim.ber.gen′ sis. M.L. *Norimberga* Nurnberg (Bavaria, Germany); M.L. fem. adj. coming from Nurnberg, referring to its place of isolation.

   As described for the genus with the following additional characteristics. No reduction of nitrate. Grows on cetrimide agar. Does not grow on acetamide; does not grow at 42°C. Assimilates citrate; does not assimilate adipate, maltose, or sucrose. Produces oxidase and phosphoamidase. Does not produce cysteine arylamidase; some strains produce urease.

   The mol% G + C of the DNA is: 63.2 (HPLC).
   Type strain: ATCC BAA-65, CCUG 39188, CIP 105463, LGM 18379.
   GenBank accession number (16S rRNA): Y09879.

3. **Pandoraea pnomenusa** Coenye, Falsen, Hoste, Ohlen, Goris, Govan, Gillis and Vandamme 2000, 896[VP]
   pno.me.nu′ sa. Gr. n. *pnoe* breath, breathing; Gr. v. *meno* to reside, stay, live; (fem. part. pres. *menusa* N.L. part. adj. *pnomenusa* referring to the lung as the niche of these bacteria.

   As for the genus with the following additional charac-
teristics. Most strains reduce nitrate. Grows at 42°C; does not grow in O/F medium with glucose. Assimilates caprate, citrate, and DL-lactate; does not assimilate adipate, D-glucose, maltose, or sucrose. Produces C$_8$-ester lipase and urease; most strains produce C$_4$-ester lipase and phosphoamidase.

   The mol% G + C of the DNA is: 64.3 (HPLC).
   Type strain: ATCC BAA-63, CIP 106626, CCUG 38742, LMG 18087.
   GenBank accession number (16S rRNA): AF139174.

4. **Pandoraea pulmonicola** Coenye, Falsen, Hoste, Ohlen, Goris, Govan, Gillis and Vandamme 2000, 896[VP]
   pul.mo.ni′ co.la. L. n. *pulmo* lung; L. suff. *cola* dwelling, occurring in; N.L. n. *pulmonicola* occurring in lungs.

   As for the genus with the following additional characteristics. No reduction of nitrate. Grows at 42°C; grows on cetrimide agar and in O/F medium with glucose. Does not grow on acetamide. Assimilates caprate, citrate, D-glucose, and DL-lactate; does not assimilate adipate, maltose, or sucrose. Produces oxidase and phosphoamidase; does not produce amylase, arginine dihydrolase, cysteine arylamidase, C$_4$-esterase, C$_8$-ester lipase, or urease.

   The mol% G + C of the DNA is: 61.8 (HPLC).
   Type strain: ATCC BAA-62, CIP 106625, CCUG 38759, LMG 18106.
   GenBank accession number (16S rRNA): AF139175.

5. **Pandoraea sputorum** Coenye, Falsen, Hoste, Ohlen, Goris, Govan, Gillis and Vandamme 2000, 896[VP]
   spu.to′ rum. L. n. *sputum* spit, sputum; L. gen. pl. n. *sputorum* of sputa.

   As for the genus with the following additional characteristics. Does not grow on acetamide or in O/F medium with glucose. Does not assimilate sucrose. Produces C$_8$-ester lipase and phosphoamidase.

   The mol% G + C of the DNA is: 61.9.
   Type strain: CCUG 39682, LMG 18819.
   GenBank accession number (16S rRNA): AF139176.

Other Organisms

Note: this description, like those of the other organisms in this section, is based on a single strain. 16S rDNA sequence analyses place all of these organisms in the genus *Pandoraea*; other data show that they are distinct from the five described species (Coenye et al., 2000; Daneshvar et al., 2001).

1. *Pandoraea* sp. (genomospecies 1). Described by Coenye et al. (2000); originally isolated by Parsons et al. (1988). This organism was studied because of its ability to degrade chlorinated aromatic compounds. Reduces nitrate. Does not grow at 42°C, in O/F medium with glucose, or on acetamide or cetrimide agar. Assimilates DL-lactate; does not assimilate adipate, caprate, or mannose. Produces C$_4$-ester lipase, C$_8$-ester lipase, oxidase, phosphoamidase, and urease.

   The mol% G + C of the DNA is: unknown.
   Deposited strain: JB1, R-5199, CCUG 39680.
   GenBank accession number (16S rRNA): X92188.

2. *Pandoraea* genomospecies 2. Described by Daneshvar et al. (2001). Does not reduce nitrate. Grows at 42°C. Does not grow on cetrimide agar. Produces oxidase; produces urease after 7 d on Christensen's agar.

   The mol% G + C of the DNA is: 65.2 (T_m).

Deposited strain: CDC G5084, ATCC BAA-108.
GenBank accession number (16S rRNA): AF247693.

3. *Pandoraea* genomospecies 3. Described by Daneshvar et al. (2001). Does not reduce nitrate. Grows at 42°C. Does not grow on cetrimide agar. Produces urease after 7 d on Christensen's agar; does not produce oxidase.
   The mol% G + C of the DNA is: 67.1 (T_m).
   Deposited strain: CDC G9805, ATCC BAA-109.

GenBank accession number (16S rRNA): AF247697.

4. *Pandoraea* genomospecies 4. Described by Daneshvar et al. (2001). Does not reduce nitrate. Grows at 42°C. Does not grow on cetrimide agar. Produces urease after 7 d on Christensen's agar; produces oxidase.
   The mol% G + C of the DNA is: 68.6 (T_m).
   Deposited strain: CDC H652, ATCC BAA-110.
   GenBank accession number (16S rRNA): AF247698.

Genus V. **Paucimonas** *Jendrossek 2001, 906*[VP]

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Pau.ci.mo'nas. L. adj. *paucus* little, few; Gr. fem. n. *monas* unit, cell; M.L. n. *Paucimonas* bacterium with restricted (few) metabolic capacities.

Gram-negative, strictly respiratory chemoorganotroph. Catalase positive; oxidase positive. **Preferred substrates are organic acids**; does not utilize alcohols or polyalcohols, amino acids or polypeptides, sugars or polysaccharides. **Accumulates poly-β-hydroxybutyrate.**

The mol% G + C of the DNA is: 59 ± 2.

Type species: **Paucimonas lemoignei** (Delafield, Doudoroff, Palleroni, Lusty and Contopoulos 1965) Jendrossek 2001, 906 (*Pseudomonas lemoignei* Delafield, Doudoroff, Palleroni, Lusty and Contopoulos 1965, 1460.)

FURTHER DESCRIPTIVE INFORMATION

Analysis of 16S rDNA sequences placed *Paucimonas lemoignei* in the *Betaproteobacteria* with *Herbaspirillum* spp. as its closest relatives (Jendrossek, 2001).

ENRICHMENT AND ISOLATION PROCEDURES

Organisms were isolated from soil. Soil samples were emended with poly-(3-hydroxyvalerate) and incubated; the soil was then diluted into a liquid enrichment medium containing poly-(3-hydroxyvalerate). Procedures and liquid and solid media are described by Mergaert et al. (1996).

List of species of the genus Paucimonas

1. **Paucimonas lemoignei** (Delafield, Doudoroff, Palleroni, Lusty and Contopoulos 1965) Jendrossek 2001, 906[VP] (*Pseudomonas lemoignei* Delafield, Doudoroff, Palleroni, Lusty and Contopoulos 1965, 1460.)
   le.moig'ne.i. M.L. gen. n. *lemoignei* of Lemoigne; named after M.H. Lemoigne, a French bacteriologist.

   Gram-negative motile rods (0.6–0.8 × 1.5–3.0 μm). Nonsporeforming; do not produce fluorescent pigments. Poly-

   hydroxyalkanoate depolymerases produced. Grow on acetate, butyrate, 3-hydroxybutyrate, 3-hydroxyvalerate, pyruvate, valerate, and succinate. Major fatty acids are $C_{12:0}$, $C_{16:0}$, $C_{16:1\ \omega7c}$, $C_{10:0\ 3OH}$, $C_{12:0\ 3OH}$, $C_{14:0\ 2OH}$.

   The mol% G + C of the DNA is: 59 ± 2 (T_m).
   Type strain: ATCC 17989, CCUG 2114, CIP 103794, DSM 7445, LMG 2207.
   GenBank accession number (16S rRNA): X92555.

Genus VI. **Polynucleobacter** *Heckmann and Schmidt 1987, 456*[VP]

HANS-DIETER GÖRTZ AND HELMUT J. SCHMIDT

Pol.y.nuc'le.o.bac.ter. Gr. adj. *polys* numerous; L. masc. n. *nucleus* nut, kernel; masc. *bacter* the equivalent of Gr. neut. n. *bactrum* a rod; *polynucleobacter* the bacterium with many nucleoids.

Bacterial endosymbiont formerly called omikron. Multiple nucleoids. Inhabits the cytoplasm of the following closely related *Euplotes* spp. (Ciliophora, Protozoa): *E. aediculatus, E. eurystomus, E. patella, E. plumipes, E. woodruffi, E. daidaleos,* and *E. octocarinatus.* Essential for their host species. Nonmotile.

The mol% G + C of the DNA is: 47.7 or 44.9.

Type species: **Polynucleobacter necessarius** Heckmann and Schmidt 1987, 456.

FURTHER DESCRIPTIVE INFORMATION

Only one species, *P. necessarius,* has been described. It has a number of features in common with other endosymbionts found in related *Euplotes* species (Heckmann et al., 1983). Approximately 900–1000 cells of *P. necessarius* inhabit the cytoplasm of *E. aediculatus* (Heckmann, 1975). If *P. necessarius* is stained with DNA-specific dyes, several intensely stained and regularly spaced dots become visible (Fig. BXII.β.4). They are considered to be nu-
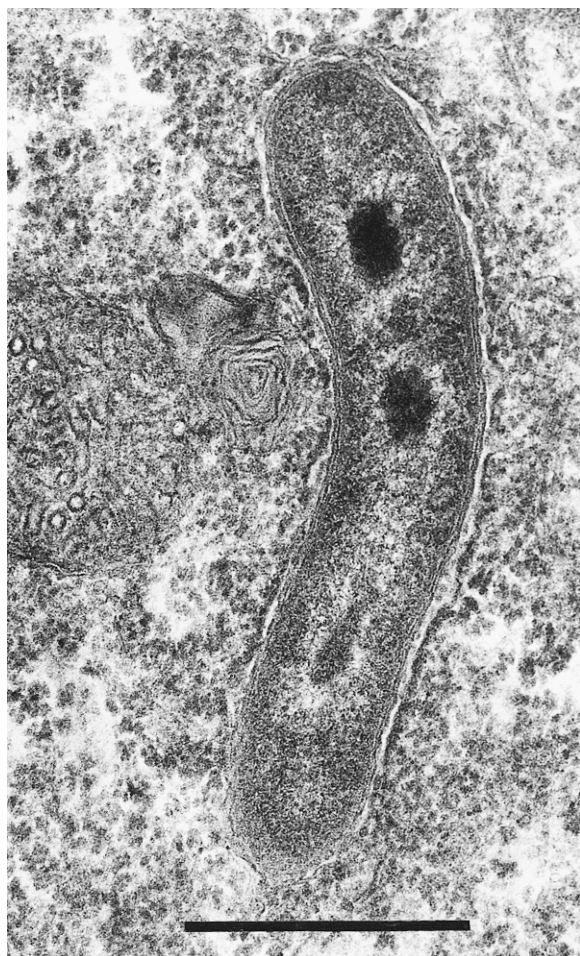
FIGURE BXII.β.4. *Polynucleobacter necessarius* (omikron), endosymbiont of *Euplotes aediculatus*. Longitudinal section showing nucleoids. Bar = 0.5 μm. (Reproduced with permission from K. Heckmann, Journal of Protozoology *22:* 97–104, 1975, ©Society of Protozoologists.)

cleoids, and differ from those of most free-living bacteria by having an electron-dense central core. The symbionts reproduce by transverse binary fission. The fission products are often found to differ from each other in size. It is possible to isolate *P. necessarius* from *E. aediculatus* homogenates and to obtain pure preparations of the symbionts in quantities large enough for an ex-

traction and characterization of their DNA (Schmidt, 1982). The data are given below under the formal description of the species.

It is possible to remove the symbionts from *E. aediculatus* by treating a rapidly growing culture with penicillin (Heckmann, 1975). Aposymbiotic hosts eventually die. Heckmann concluded that *P. necessarius* is essential for the life of its host. Fauré-Fremiet (1952) made the same observation for *E. patella* and *E. eurystomus* and reached the same conclusions.

TAXONOMIC COMMENTS

Only the best-studied endosymbiont of *Euplotes*, *P. necessarius*, has been assigned a binomial name. This endosymbiont, previously known as omikron, lives obligately in *Euplotes aediculatus* and was named *Polynucleobacter necessarius* by Heckmann and Schmidt (1987). Similar, still unnamed endosymbionts inhabit related species of *Euplotes*, and are called omikron-like endosymbionts.

P. necessarius belongs to the *Betaproteobacteria* and is rather closely related to *Ralstonia eutrophus*, *R. solanacearum*, and *R. pickettii* (Fig. BXII.β.5). *In situ* hybridization with a specific oligonucleotide probe corroborated the assignment of the retrieved species to *P. necessarius* (Springer et al., 1996).

Omikron-like symbionts occur in several closely related species of freshwater *Euplotes* (*E. aediculatus*, *E. eurystomus*, *E. patella*, *E. plumipes*, *E. woodruffi*, *E. daidaleos*, and *E. octocarinatus*), but not in other unrelated freshwater species. In many stocks of these species, penicillin treatment has been found to remove the symbionts, which affected their hosts as described above, eventually resulting in their death (Heckmann et al., 1983; Heckmann, 1983). In this connection, it is of interest that Foissner (1977) described a species (*E. moebiusi f. quadricirratus* Kahl, 1930) in which he found omikron-like symbionts. This species is not closely related to the above *Euplotes* species. Foissner has not tested, however, whether or not the ciliate depends upon the symbionts. Fujishima and Heckmann (1984) tried to transfer symbionts between *Euplotes* species. They produced aposymbiotic cells by treating them with penicillin, and observed restoration of growth and the ability to divide when symbionts of *E. woodruffi*, of a stock collected in Japan, were introduced into a stock of *E. aediculatus*, which had been collected in France. The reverse combination, however, with *E. woodruffi* as the recipient and *E. aecliculatus* as the donor, did not work.

FURTHER READING

Heckmann, K. 1983. Endosymbionts of *Euplotes*. Int. Rev. Cytol. Suppl. *14*: 111–114.

List of species of the genus Polynucleobacter

1. **Polynucleobacter necessarius** Heckmann and Schmidt 1987, 456[VP]

   nec.es.sar′ i.us. L. adj. *necessarius* indispensable, necessary.

   Description as given for the genus. Three to twelve nucleoids. Cells are 0.3 × 2.5–7.5 μm. Divides by binary fission.

DNA molecular weight 3.5×10^9 daltons (analytic complexity) or 0.57×10^9 daltons (kinetic complexity).

The mol% G + C of the DNA is: 47.7 (T_m) and 44.9 (Bd).

Type strain: ATCC 30859.

GenBank accession number (16S rRNA): X93019.

Additional Remarks: The type strain is isolated from stock 15 of *E. aediculatus* (ATCC 30859).

Other Organisms

Other bacterial endosymbionts found in *Euplotes* have not been given binomial names, nor have they been validly described. Several endosymbionts of *Euplotes* produce the killer phenotype, and strains of killers with associated bacteria in the cytoplasm have been described for *E. minuta* and *E. crassus*. They were

named epsilon and eta, respectively, by Heckmann et al. (1967) and Rosati et al. (1976).

Certain stocks of *E. patella*, collected in Japan, were reported to be mate killers by Katashima (1965). Mate killers kill sensitive ciliates in the act of conjugation, when toxins produced by en-
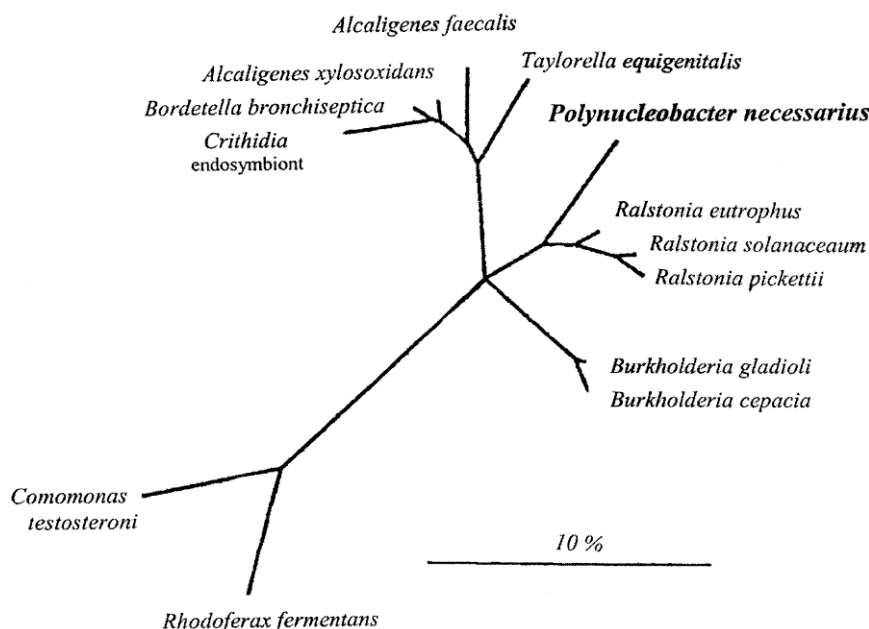
FIGURE BXII.β.5. Phylogenetic tree reflecting the relationship of *Polynucleobacter necessarius* and selected members of the *Betaproteobacteria*. The tree is based on a maximum likelihood analysis of a data set comprising a selection of 75 16S rRNA gene sequences containing no more than 140 ambiguities (for details see Springer et al., 1996) (Reproduced with permission from N. Springer et al., FEMS Microbiology Letters *135:* 333–336, 1996 ©Elsevier B.)

dosymbionts of mate killers are thought to be transferred to the sensitive mating partner not bearing endosymbionts. Mate killing was also observed for *E. minuta* by Heckmann et al. (1967) and for *E. crassus* by Rosati and Verni (1977). All these mate killers contained unnamed endosymbionts in their cytoplasm. *E. crassus* sometimes contains other endosymbionts with no known effects on their hosts. Rosati et al. (1976) observed them mostly in the cytoplasm. However, Rosati and Verni (1975) did find one endosymbiont in the macronucleus.

Genus VII. **Ralstonia** Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1996, 625[VP] (Effective publication: Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1995, 902)

EIKO YABUUCHI, YOSHIAKI KAWAMURA AND TAKAYUKI EZAKI

Ral.sto′ n.ia. M.L. dim. *-ia* ending; M.L. fem. n. *Ralstonia* named after E. Ralston, the American bacteriologist who first described *Pseudomonas pickettii*.

Gram-negative asporogenous rods. Motile or nonmotile; motile species have either a single polar flagellum or peritrichous flagella. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Able to grow on ordinary peptone media. One species, *R. eutropha*, is a facultatively chemolithoautotrophic organism (a knallgas bacterium) and can oxidize H_2 as an electron donor. Furthermore, under anaerobic conditions, *R. eutropha* is able to utilize nitrate as the terminal electron acceptor. Colony color is beige in most species. Strains of *R. eutropha* are regarded as having outstanding biotechnological potential. **Oxidase and catalase positive.** Lysine and ornithine decarboxylase negative. Ubiquinone Q-8 is the major respiratory quinone. **None of the 26 carbohydrates tested are oxidized by the type strains of *R. campinensis* and *R. taiwanensis.*** Among 95 other organic compounds, 10—mainly salts of organic acids—were assimilated and 39 other compounds were not assimilated by all of the 11 type strains. **Cellular lipids of this genus contain two kinds of phosphatidylethanolamine, PE-1 and PE-2. The latter possesses 2-hydroxy fatty acid at *sn*-2 position of the glycerol moiety.** Major components of cellular fatty acids are $C_{16:0}$, a mixture of $C_{18:1\ \omega 9t}$ and $C_{18:1\ \omega 7c}$, and $C_{14:0\ 3OH}$. At present the genus is composed of 11 validated species. The sequence similarity of 11 type strains to that of *Ralstonia pickettii*—the type species—ranges from 95.0% to 98.1%. The genus contains **plant pathogens, human pathogens, knallgas bacteria, and metal-resistant bacteria**.

The mol% G + C of the DNA is: 64.0–68.0.

Type species: **Ralstonia pickettii** (Ralston, Palleroni and Doudoroff 1973) Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1996, 625 (Effective publication: Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1995, 903) (*Burkholderia pickettii* Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398; *Pseudomonas pickettii* Ralston, Palleroni and Doudoroff 1973, 18.)

FURTHER DESCRIPTIVE INFORMATION

Morphology The cell dimensions are 0.5–0.8 × 1.2–3.0 μm, except for *R. oxalatica*, whose cells are as small as 0.3–0.4 × 0.9–1.5 μm.

Poly-β-hydroxybutyrate (PHB) granules have been reported in both Gram-negative and Gram-positive bacteria as intracellular reserve material (Doudoroff and Stanier, 1959). Morphological detection of PHB granules by optical or fluorescent microscopy has been reported (Stanier et al., 1966; Ostle and Holt, 1982). Rapid detection of polyhydroxyalkanoate-accumulating bacteria isolated from the environment by colony PCR has been reported by Sheu et al. (2000). *R. eutropha* synthesizes short chain length (SCL; 3–5 carbon atoms) poly-β-hydroxyalkanoates (PHAs), and the resulting polymers are accumulated as intracellular granules. The synthesis of the PHAs is catalyzed by PHA synthase (Song et al., 2000c; Zhang et al., 2000c). PHAs are now of commercial value as thermoplastics (Holmes, 1985). Accumulated PHB is degraded and used for growth and survival when an exogenous carbon source is not available (Handrick et al., 2000). Compared with PHB homopolymers, the PHA copolymer (poly-β-hydroxybutyrate-co-β-hydroxyvalerate) [P(HB)-co-HV)] has more useful thermomechanical properties. *R. eutropha* accumulates larger amount of [P(HB)-co-HV] copolymer when provided with glucose and pentanoic acid rather than propionic acid under nitrogen-limited conditions (Ramsay et al., 1990).

Cloning of the gene encoding PHB polymerase and its expression by recombinant *E. coli* has been reported (Schubert et al., 1988). The PHB production by the recombinant *E. coli* was analyzed metabolically and kinetically (van Wegen et al., 2001). An intracellular PHB depolymerase gene from *R. eutropha* H16 was cloned and gene product was characterized (Saegusa et al., 2001).

Colonial morphology and pigmentation Colonies on tryptic soy agar are less than 1 mm in diameter after 48 h incubation at 28–30°C. When the colonies are fully-grown, their diameter is >1 mm. In six species, the colonies are beige-colored, domed, smooth, and glistening with entire margin. The colonies of two species (*R. campinensis* and *R. metallidurans*) sometimes have scalloped margins (Goris et al., 2001). *R. solanacearum* EY 2181 produces a water-soluble slightly brown pigment, but the color appears different from that of alcaptone.

Cells of *R. solanacearum* strain 1609 convert to a viable-but-nonculturable (VBNC) form in water microcosms kept at 4°C, but not in those at 20°C (van Elsas et al., 2001). The viability of a fraction of these VBNC forms was evidenced by the direct viable count staining with the redox dye 5-cyano-2,3-ditolyl tetrazolium chloride (van Elsas et al., 2001). Recently, it was reported that cells of *R. solanacearum* enter into a VBNC state under certain conditions, as in response to cupric sulfate when in a saline solution, and when placed in autoclaved soil (Grey and Steck, 2001). It was also verified that *R. solanacearum* cells in the VBNC state were able to infect and multiply in the tissue. Thus the VBNC form of plant pathogens might explain the persistence of infection in nature. The occurrence of VBNC cells of *R. solanacearum* in natural water poses new problems for the detection (van Elsas et al., 2001) and persistence of infection (Grey and Steck, 2001) of *R. solanacearum*.

Growth conditions and nutrition Growth occurs aerobically on ordinary peptone media at 25–41°C. The type strains of *R. basilensis*, *R. metallidurans*, and *R. solanacearum* do not grow at 41°C, but the type strains of the other species can do so. The organisms do not require any growth factors, including sodium chloride. *Ralstonia eutropha* is a H_2-oxidizing (knallgas) chemolithoautotroph (Aragno and Schlegel, 1992); [NiFe] hydrogenases play a role as H_2 sensors (Kleihues et al., 2000; Buhrke et al., 2001). Cells of *R. eutropha* also grow well aerobically on nutrient-rich ordinary media.

Physiology and metabolism *Ralstonia eutropha* strain H16 mediates the reduction of nitric oxide (NO) to nitrous oxide (N_2O) by a single-component nitric oxide reductase (Cramm et al., 1999; Pohlmann et al., 2000). Resistance to nickel (Schmidt et al., 1991) and to both cobalt and nickel (Tibazarwa et al., 2000) has been reported in *R. eutropha*. Lead resistance was reported in *R. metallidurans* (Borremans et al., 2001). Genetically engineered *R. eutropha* strain MTB has an enhanced ability to immobilize external Cd^{2+} ions, and inoculation of this strain into Cd^{2+}-polluted soil reduces the toxic effects of heavy metal on the growth of tobacco plants (*Nicotiana bentamiana*) (Valls et al., 2000).

Recently, many reports concerning metabolic activities of *R. eutropha* have appeared in the literature (Lütke-Eversloh and Steinbuchel, 1999; Grzeszik et al., 2000; Happe et al., 2000; Padilla et al., 2000; Bernhard et al., 2001; Bramer and Steinbuchel, 2001; Drewlo et al., 2001; Schräder et al., 2001; Taguchi et al., 2001; York et al., 2001; Zarnt et al., 2001). The depolymerase gene for intracellular PHB of *R. eutropha* strain H16 was successfully cloned and its product was characterized (Saegusa et al., 2001). Under anaerobic conditions, *R. eutropha* undergoes nitrate respiration by utilizing nitrate as a terminal electron acceptor (Schwartz and Friedrich, 2001). Genetic determinants essential for these metabolic processes are linked to the megaplasmid pHG1.

Cellular lipids and fatty acids composition All of the two-dimensional TLCs (thin-layer chromatograms) of the type strains of eight *Ralstonia* species and of *Burkholderia cepacia* (Fig. BXII.β.6) reveal two spots of phosphatidylethanolamine (PE-1 and PE-2) and one spot of phosphatidylglycerol (PG). PE-2 possesses 2-hydroxy fatty acid, at the sn-2 position of the glycerol moiety. In addition, two spots of ornithine lipid (OL-1 and OL-2) have been visualized in *B. cepacia*. The major components of the nonpolar acids are $C_{16:0}$ followed by $C_{16:1\ \omega7c}$ and mixture of $C_{18:1\ \omega9t}$ and $C_{18:1\ \omega7c}$. The major polar acid is $C_{14:0\ 3OH}$. Percentage of minor components of fatty acids against the total amount of each of them cannot be a key of differentiation of species, because it could vary by differences in cultural condition and/or analyzing method.

Genetics and plasmids The complete genome sequence and its analysis of *R. solanacearum* strain GMI100 have been reported (Salanoubat et al., 2002). Part of their summary is as follows: "The 5.8-megabase (Mb) genome is organized into two replicons, one 3.7-Mb chromosome and another 2.1-Mb megaplasmid. Both replicons have a mosaic structure providing evidence for the acquisition of genes through horizontal gene transfer. Regions containing genetically mobile elements associated with the percentage of G + C bias may have an important function in genome evolution. The genome encodes many proteins potentially associated with a role in pathogenicity. In particular, many putative attachment factors were identified. The complete repertoire of type III secreted effector proteins can be studied. Over 40 candidates were identified. Comparison with other genomes
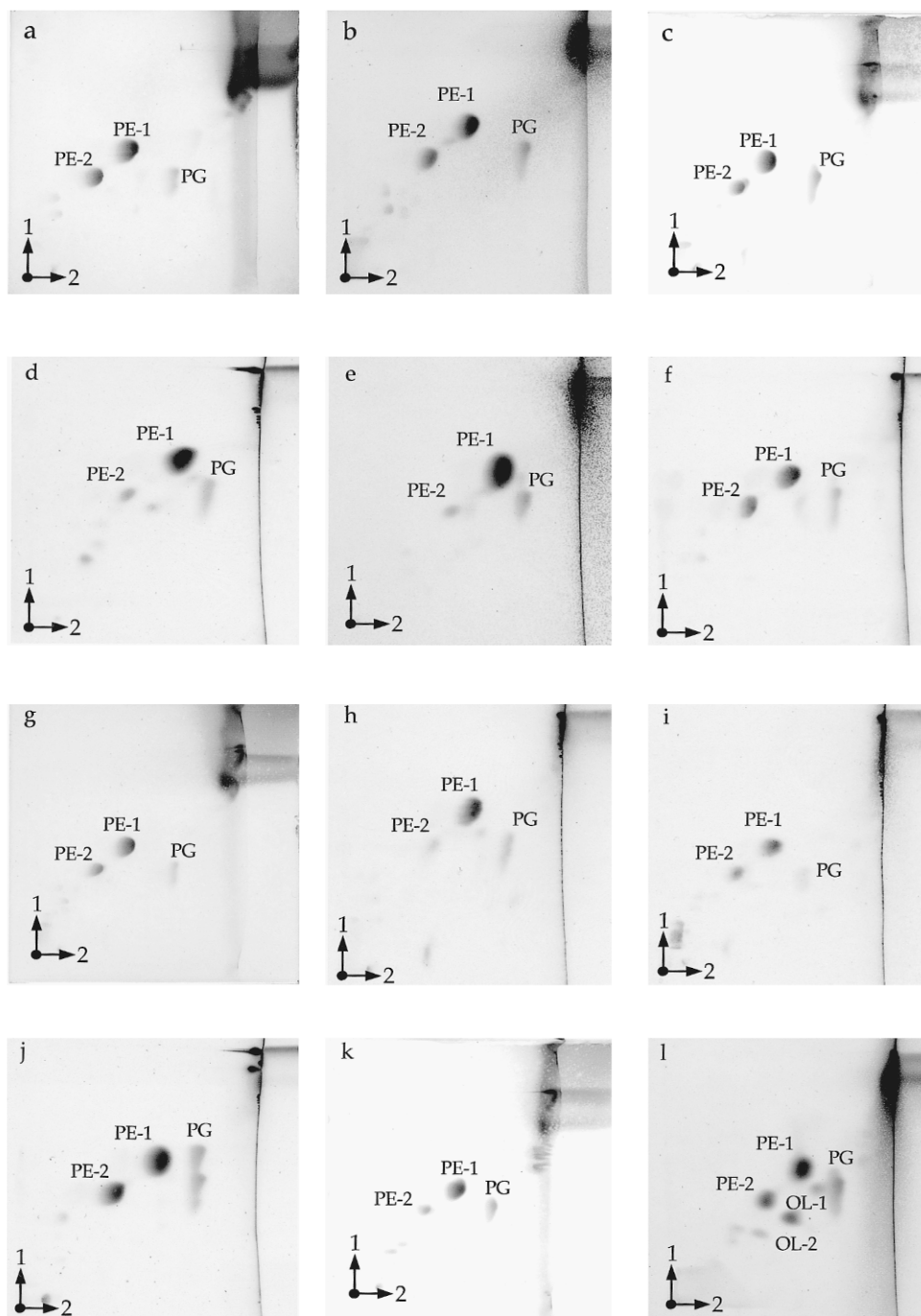
FIGURE BXII.β.6. Two-dimensional thin-layer chromatograms of cellular lipids of type strains of eight *Ralstonia* species and of *Burkholderia cepacia* (N. Fujiwara and T. Naka, unpublished data). The solvent system for the first direction was chloroform:methanol:water (65:25:4, v/v); for the second direction it was chloroform:methanol:acetic acid (65:25:10, v/v). (*a*) *R. pickettii* EY 4382; (*b*) *R. basilensis* EY4358; (*c*) *R. campinensis* EY 4379 (*d*) *R. eutropha* EY 3798; (*e*) *R. gilardii* EY 4363; (*f*) *R. mannitolilytica* EY 4364; (*g*) *R. metallidurans* EY 4380; (*h*) *R. oxalatica* EY 4365; (*i*) *R. paucula* EY 4366; (*j*) *R. solanacearum* EY 2181; (*k*) *R. taiwanensis* EY 4381; (*l*) *B. cepacia* EY 645. Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; OL, ornithine lipid.

suggests that bacterial plant pathogens and animal pathogens harbor distinct arrays of specialized type III-dependent effectors."

The genome sequence of *R. solanacearum* is a first step toward an exhaustive functional analysis of pathogenicity determinants in this plant pathogen. Prior to this report, much genetic research on the virulence factors of *R. solanacearum* was reported (Rosenberg et al., 1982; Boucher et al., 1986; Allen et al., 1997; Huang and Allen, 1997; Lasserre et al., 1997; Huang et al., 1998; Aldon et al., 2000; Bertolla et al., 2000; Garg et al., 2000; Vasse et al., 2000; Wang et al., 2000; Belbahri et al., 2001; Schwartz and Friedrich, 2001; Staskawicz et al., 2001; van Overbeek et al., 2002).

The genome of *R. eutropha* strain H16 is composed of two circular chromosomes measuring 4.1 and 2.9 Mb. A physical map of the megaplasmid pHG1 has been given by Schwartz and Fiedrich (2001).

A bacteriophage P4284 encoding a *R. solanacearum* bacteriolytic protein has been described (Ozawa et al., 2001).

Pathogenicity   The plant pathogens produce a pilus structure. Morphologically similar structures are associated with type III protein secretion systems found in animal pathogens, and are essential for the delivery of virulence proteins directly into host cells.

Ralstonia solanacearum causes a lethal vascular wilt disease in more than 200 plant species in 50 botanical families in the tropics, subtropics, and warm temperate regions of the world. Its agronomically important hosts include tomato, potato, tobacco, peanut, and banana. This pathogenic organism was named and described as early as 1896 by Smith (1896). The instability of virulence and correlation with colony morphology of this organism led to elucidation of the existence of a regulatory network modulating transcription of multiple virulence genes (Brumbley et al., 1993). A type IV pilus system is responsible for a twitching type of motility and seems to contribute significantly to the plant pathogeneses (Liu et al., 2001; Tans-Kersten et al., 2001). After *R. solanacearum* cells penetrate the xylem vessels of tomato plants, they rapidly travel to the upper part of the plant and become readily detectable throughout the stem. Viable counts of the organism can reach $>10^{10}$ cells per cm of stem by day 8. Infected plants rapidly collapse and die, and virulent organisms released from the host plant roots or collapsed stems in contact with the ground results in a return of the bacteria to a saprophytic life in the soil, where they await a new host (Schell, 2000). The primary virulence factor of wild type *R. solanacearum* is an exopolysaccharide I (EPS I)—a large, nitrogen-rich, acidic exopolysaccharide that occludes vascular tissues and inhibits water flow (Schell, 2000). A type II secretion system exports all the cell wall-degrading exoenzymes such as pectinolytic enzymes and cellulolytic enzymes (Saile et al., 1997). A type III secretion system seems to deliver toxic proteins directly into the plant cell cytoplasm. Production of several virulence determinants is governed by growth in the presence of a volatile extracellular factor (VEF) produced by the wild-type strain of the organism (Clough et al., 1994). The VEF is active at ≤1 nM for stimulating biosynthesis of extracellular polysaccharide (*eps*) (Flavier et al., 1997a). The virulence of *R. solanacearum* is regulated by a complex network, the core of which is the Phc (phenotype conversion) system (Schell, 2000). The PhcA system is composed of PhcA (Brumbley and Denny, 1990; Brumbley et al., 1993) and the products of the *phcBSRQ* operon (Clough et al., 1994, 1997). Transcriptional activity of PhcA is controlled by 3-OH PAME (Clough et al., 1997; Flavier et al. 1997b).

Three *Ralstonia* species infect predisposed humans: *R. pickettii*, *R. mannitolilytica*, and *R. gilardii*. *R. pickettii* has been associated with acute meningitis (Fass and Barnishan, 1976), osteomyelitis and intervertebral discitis (Wertheim and Markovitz, 1992), nosocomial infection (Phillips et al.; 1972; Kahan et al., 1983; Raveh et al., 1993; Maroye et al., 2000), bacteremia (Fujita et al., 1981; Roberts et al., 1990), pseudobacteremia (Verschraegen et al., 1985), and colonization (McNeil et al., 1985; Centers for Disease Control and Prevention, 1998; Labarca et al., 1999; Yoneyama et al., 2000). One case each of recurrent meningitis and hemoperitoneum infection due to *R. mannitolilytica* (Vaneechoutte et al., 2001) has been reported. In addition, a case of catheter sepsis associated with *R. gilardii* (Wauters et al., 2001) has been reported.

It is remarkable that a certain strain of *Pseudomonas aeruginosa* is not only pathogenic to predisposed humans but also capable of causing disease in plants. Such "interkingdom" pathogens are able to colonize the surface of both plant and animal cells and to escape from diverse host defense mechanisms. Such features that allow them to be pathogenic on both procaryotic and eucaryotic hosts should be elucidated in the future (Staskawicz et al., 2001).

Ecology   Organisms belonging to genus *Ralstonia* appear to be free-living in nature. However, because of their metabolic or pathogenic specialties, the type strains of certain species have been isolated from human clinical specimens (*R. pickettii*, *R. mannitolilytica*, and *R. paucula*), from a fixed-bed reactor with 2,6-dichlorophenol as the sole carbon and energy source (*R. basilensis*), from a zinc-decertified area in Lommel, Belgium (*R. campinensis*), from water (*R. eutropha* and *R. metallidurans*), from plants (*R. solanacearum* and *R. taiwanensis*), and from earthworms (*R. oxalatica*). Thus, *Ralstonia* organisms might be living in a variety of ecological niches. Furthermore, the long survival of *R. solanacearum* organisms in soil and rhizospheres might be possible by an asymptomatic infection of the roots of nonhost plants, such as bean, peas, soybean, corn, and rice (Granada and Sequeira, 1983).

DIFFERENTIATION OF THE GENUS *RALSTONIA* FROM OTHER GENERA

Differential characteristics of the type species of five genera in the order *Burkholderiales* (class *Betaproteobacteria*) with one genus each in the order *Sphingomonadales* and *Caulobacterales* (class *Alphaproteobacteria*) and the type genus of the order *Pseudomonadales* (class *Gammaproteobacteria*) are summarized in Table BXII.β.8.

TAXONOMIC COMMENTS

The genus *Ralstonia* was named after Ericka Ralston, the American bacteriologist who first named and described *Pseudomonas pickettii* and suggested a taxonomic relationship to *Pseudomonas solanacearum* (Ralston et al., 1973). Based on polyphasic taxonomic research, *Burkholderia pickettii*, *Burkholderia solanacearum*, and *Alcaligenes eutrophus* were transferred to a new genus, *Ralstonia*, by Yabuuchi et al. (1995, 1996). *R. pickettii* was designated as the type species for the genus, because neither plant nor animal quarantine law restricts its exchange. This is important for taxonomic research to proceed smoothly. The genus *Ralstonia*, when proposed, contained *R. pickettii*, a species pathogenic to predisposed humans and pathogenic to plants in the botanical

TABLE BXII.β.8. Differential characteristics of the type species of *Ralstonia* and the type species of seven other genera of *Proteobacteria*[a]

Characteristic	*Ralstonia pickettii*	*Burkholderia cepacia*	*Oxalobacter formigenes*	*Alcaligenes faecalis*[b]	*Comamonas terrigena*[c]	*Sphingomonas paucimobilis*	*Brevundimonas diminuta*[d]	*Pseudomonas aeruginosa*[e]
Classification in:								
Betaproteobacteria	+	+	+	+	+	−	−	−
Alphaproteobacteria	−	−	−	−	−	+	+	−
Gammaproteobacteria	−	−	−	−	−	−	−	+
Aerobic	+	+	−	+	+	+	+	+
Anaerobic	−	−	+	−	−	−	−	−
Flagellation:								
Single polar	+	−	−	−	−	+	+	+
Polar tuft	−	+	−	−	+	−	−	−
Peritrichous	−	−	−	+	−	−	−	−
Pigmentation:								
of colony	Beige	Yellow	−	Iridescent	−	Yellow	−	−
of medium	−	−	Clearance of oxalate crystals	−	−	−	−	Blue/green
Growth factor required	−	−	Rumen fluid or yeast extract	−	−		+	−
Denitrification	+	−				−		+
Esculin hydrolysis	−	+		−	−	+	−	−
Lysine decarboxylase	−	+		−	−	−	−	−
Urease	+	−		−	−	−		−
Oxidative formation of acid from glucose	+	+	−	−	−	+	−	+
Assimilation of:								
Glucose	+	+		−		+	−	+
Citrate	+	+		+	−	+	−	+
Sphingolipid	−	−		−		+		−
Characteristic hydroxy fatty acid:								
$C_{10:0\ 3OH}$					+			
$C_{12:0\ 2OH}$								+
$C_{12:0\ 3OH}$							+	+
$C_{14:0\ 2OH}$						+		
$C_{14:0\ 3OH}$	+	+		+	+			
$C_{16:0\ 3OH}$		+						
Isoprenoid quinone:								
Q-8	+	+		+	+	+		
Q-9								+
Q-10							+	
Mol% G + C of DNA	64	66.6	48–51	55.9–59.4[d]	64–66[c]	62.1–63.9	79.3–81.4	67.2[g]
Pathogenic for:								
Humans	+						+	+
Mammals		+						
Plants								+
Regulates homeostasis of oxalic acid in gut			+[c]					

[a]Symbols: see standard definitions. Blank space, not determined or not applicable.

[b]Data from Kersters and De Ley (1984b).

[c]Data from De Vos et al. (1985b).

[d]Data from Segers et al. (1994).

[e]Data from Palleroni (1984).

family Soranaceae. The genus also contained *R. solanacearum* and the hydrogen-oxidizing knallgas bacterium, *R. eutropha*. Eight new species were also added.

A phylogenetic dendrogram of the 16S rDNA similarities of the type strains of 11 *Ralstonia* species and of *Burkholderia cepacia* is shown in Fig. BXII.β.7. The percent similarity values among the type strains of 10 *Ralstonia* species and *B. cepacia* versus the type strain of *R. pickettii* are summarized in Table BXII.β.9. The values range from 95.0% to 98.1% among the *Ralstonia* species and 91.3% between *R. pickettii* and *B. cepacia*. In view of the latter value, inclusion of the genus *Ralstonia* in the family *Burkholderiaceae* is supported.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *RALSTONIA*

Tables BXII.β.10 and BXII.β.11 list the differential characteristics of the species of *Ralstonia* and of *Burkholderia cepacia*. Additional features are listed in Tables BXII.β.12, BXII.β.13, and BXII.β.14.

List of species of the genus Ralstonia

1. **Ralstonia pickettii** (Ralston, Palleroni and Doudoroff 1973) Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1996, 625[VP] (Effective publication: Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1995, 903) (*Burkholderia pickettii* Yabuuchi, Ko-
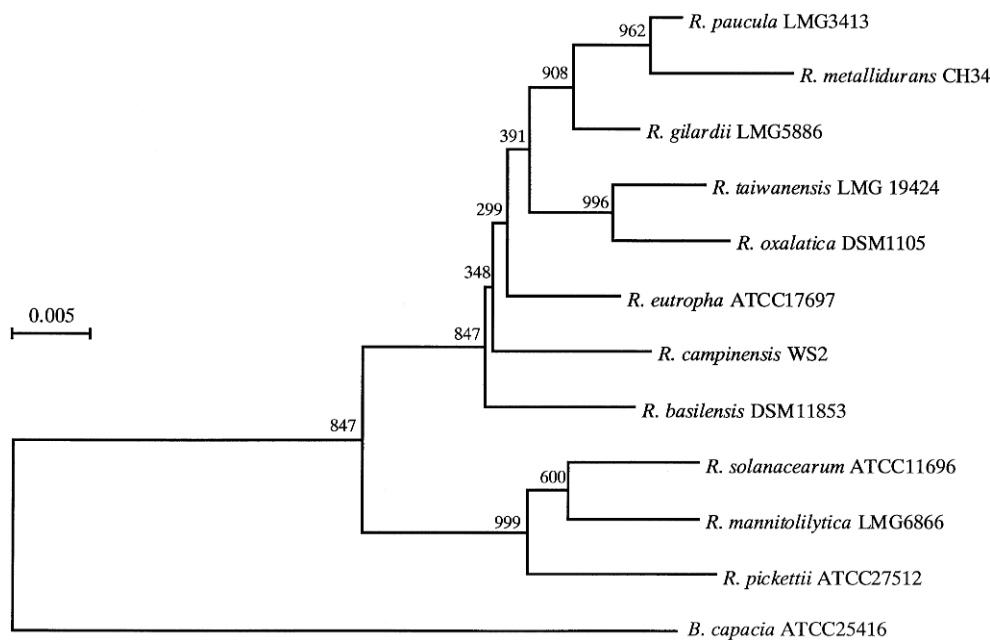
FIGURE BXII.β.7. Phylogenetic tree derived from 16S rDNA sequence analysis showing the relationships among the 11 *Ralstonia* species. Distance of the type species of the genus *Burkholderia*, *B. cepacia*, from the genus *Ralstonia* is also shown. Bar = K$_{nuc}$ value.

TABLE BXII.β.9. 16S rDNA nucleotide sequence similarity of the type strains of 10 *Ralstonia* species and of *Burkholderia cepacia* vs. that of *Ralstonia pickettii*

Species	Type stain	Nucleotide sequence accession No.	% Sequence similarity to that of ATCC 27511
R. pickettii	ATCC 27511	S55004	100
R. mannitolilytica	LMG 6866	AJ270258	98.1
R. solanacearum	ATCC 10696	X67036	97.3
R. campinensis	WS2	AF312020	96.4
R. eutropha	ATCC 17697	M32021	96.4
R. gilardii	LMG 5886	AF076645	95.9
R. taiwanensis	LMG 19425	AF300325	95.7
R. paucula	LMG 3413	AF085226	95.6
R. basilensis	DSM 11853	AF312022	95.4
R. metallidurans	CH34	Yl0824	95.3
R. oxalatica	Ox1, DSM 1105	AF155567	95
Burkholderia cepacia	ATCC 25416	M22518	91.3

sako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa 1993, 398; *Pseudomonas pickettii* Ralston, Palleroni and Doudoroff 1973, 18.)

pick.et′ ti.i. M.L. gen. n. *pickettii* Pickett patronymic, of Pickett; named after M.J. Pickett.

The characteristics are as described for the genus and in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. The cell size is 0.5–0.6 × 1.5–3.0 μm. Accumulate poly-β-hydroxy-butyrate granules as a reserve carbon source (Ralston et al., 1973). Growth occurs at 41°C but not at 5°C. Nitrate respiration is positive. Cellular lipids of the type strain contain two kinds of ornithine lipids (Fig. BXII.β.6). The former CDC group Va-2 as well as Va-1 corresponds to *R. pickettii*, because the DNA–DNA hybridization value between type strain of *R. pickettii* and Va-2 strain was 84% (Pickett and Greenwood, 1980). The type strain was isolated from a patient who had undergone tracheostomy.

The mol% G + C of the DNA is: 64.0 (Bd).

Type strain: Ralston K-288, ATCC 27511, CCUG 3318, DSM 6297, EY 4382, GTC 1882, JCM 5969, LMG 5942.

GenBank accession number (16S rRNA): S55004, X67042.

2. **Ralstonia basilensis** Steinle, Stucki, Stettler and Hanselmann 1999, 1325^{VP} (Effective publication: Steinle, Stucki, Stettler and Hanselmann 1998, 2569) emend. Goris, De Vos, Coenye, Host, Janssens, Brim, Diels, Mergeay, Kersters and Vandamme 2001, 1781.

ba.si.len′ sis. M.L. adj. *basilea, basilensis* pertaining to Basel, Switzerland, where the strain was isolated.

The characteristics are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. The cell size is 0.8 × 1.2–2.2 μm (Goris et al., 2001). Able to grow utilizing 2,6-dichlorophenol as the sole source of carbon and energy. The type strain was isolated from a fixed-bed reactor with 2,6-dichlorophenol as the sole carbon and energy source.

The mol% G + C of the DNA is: 65.0 (HPLC).

TABLE BXII.β.10. Differential physiological and biochemical characteristics of the type strains of 11 *Ralstonia* species and the type strain of *Burkholderia cepacia*[a]

Substrate or test	*R. pickettii* EY 4382	*R. basilensis* EY 4368	*R. campinensis* EY 4379	*R. eutropha* EY 3798	*R. gilardii* EY4363	*R. mannitolilytica* EY 4364	*R. metallidurans* EY 4380	*R. oxalatica* EY 4365	*R. paucula* EY 4366	*R. solanacearum* EY 2181	*R. taiwanensis* EY 4381	*Burkholderia cepacia* EY 645
Motility	+	−	+	+	+	+	+	+	+	−		+
Flagellation:												
Single polar	+			−	+	+		+	−			−
Polar tuft	−			−	−	−		−	−		+	+
Peritrichous	−			+	−	−		−	+			−
Growth at 41°C	+		+	+	+	+	−	+	+		+	−
Colony pigmentation	Beige	Beige			Beige	Beige	Beige	Beige	Beige		Beige	Yellow
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
Citrate (Simmons)	+	+	+	+	−	+	+	+	+	+	+	+
Nitrate to gas	+	−	+	−	−	+	+	+	−	−	−	−
Nitrite test	+	−	−	+	+	−	+	−	−	+	−	+
Zn test on negative nitrite test		+	−	−	+	+	−	+	+	−	+	−
Hydrolysis of:												
Esculin	+	−	−	−	−	−	−	−	−	−	−	+
Gelatin	+	−	−	−	−	−	−	−	−	+	−	+
Starch	−	−	−	−	−	−	−	−	−	−	−	−
Tween 80	−	−	−	+	−	+	−	−	−	+	+	+
Lysine decarboxylase	−	−	−	−	−	−	−	−	−	−	−	+
Phenylalanine deaminase	+	−	−	−	−	−	−	−	−	−	−	−
Urease	+	−	+	−	−	−	−	−	−	+	−	+
Acylamidase	−	−	−	−	−	−	−	−	+	−	−	−
Oxidative acid formed in OF base medium containing:												
Adonitol	−	−	−	−	−	−	−	−	−	−	−	+
Salicin	−	+	−	+	−	−	−	−	−	−	−	+
D-Ribose	+	−	−	+	−	−	−	−	−	−	−	+
Inositol	−	−	−	−	−	−	−	−	−	−	−	+
Mannitol	−	−	−	−	−	+	−	+	−	−	−	+
Sorbitol	−	−	−	−	−	−	−	+	−	−	−	+
L-Arabinose	−	−	−	−	+	+	−	+	+	−	−	+
Mannose	+	−	−	+	+	+	−	+	+	+	−	+
Dulcitol, D-arabinose	−	−	−	−	−	−	−	−	−	−	−	−
Raffinose, rhamnose	−	−	−	−	−	+	−	+	−	−	−	+
Melezitose	−	−	−	−	−	+	+	+	−	−	−	+
Melibiose	−	−	−	−	−	+	−	−	−	−	−	−
Inulin	−	−	−	+	+	+	−	−	−	−	−	−
Sucrose, trehalose	−	−	−	−	−	−	−	−	−	+	−	+
Maltose	+	−	−	−	−	−	−	+	−	+	−	+
Cellobiose	+	−	−	+	−	−	−	+	−	−	−	+
Glucose	+	+	−	+	+	+	+	+	−	−	−	+
Galactose	+	+	−	+	+	+	−	−	−	+	−	+
Lactose	−	−	−	+	+	+	−	−	−	+	−	+
Ethanol (3%)	+	+	−	−	−	+	−	+	−	+	−	+
Glycerol, xylose	+	−	−	+	−	+	−	+	−	+	−	+
Fructose	+	+	−	+	−	+	−	+	−	+	−	+
Alkaline reaction in OF base medium	+	+	−		+	+	−	+	+	+	−	+
Major respiratory quinone	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8
Mol% G + C of DNA	64	65.4	66.6	66.5	67.2	66.2	63.7	68	63.7	66.5	67.3	66.6

[a]Symbols: +, positive; −, negative; blank space, not determined or not applicable.

TABLE BXII.β.11. Results of API 20 NE of type strains of 11 *Ralstonia* species and of *Burkholderia cepacia* [a]

Substrate or test	*Ralstonia pickettii* EY 4382[T]	*Ralstonia basilensis* EY 4368[T]	*Ralstonia campinensis* EY 4379[T]	*Ralstonia eutropha* EY 3798[T]	*Ralstonia gilardii* EY 4363[T]	*Ralstonia mannitolilytica* EY 4364[T]	*Ralstonia metallidurans* EY 4380[T]	*Ralstonia oxalatica* EY 4365[T]	*Ralstonia paucula* EY 4366[T]	*Ralstonia solanacearum* EY 2181[T]	*Ralstonia taiwanensis* EY 4381[Tb]	*Burkholderia cepacia* EY 645[T]
Urea	−	−	−	−	−	−	−	−	−	−	−	−
Esculin	−	+	−	−	−	−	−	−	+	+	+	+
p-Nitro-β-D-galactopyranoside	−	−	−	−	−	−	−	−	−	−	−	+
Assimilation of:												
Glucose	+	−	−	−	−	+	−	−	−	+	−	+
D-Mannitol	−	−	−	−	−	+	−	−	−	−	+	+
Maltose	+	−	−	−	−	−	−	+	−	−	−	−
Potassium gluconate	−	+	+	+	+	+	+	+	+	−	+	+
n-Caprate	+	+	+	+	+	+	+	+	+	−	+	+
Adipate	+	+	+	+	−	−	+	+	+	−	+	+
Sodium citrate	−	+	−	+	−	+	+	+	+	+	−	+
Phenylacetate	+	+	+	+	+	+	+	+	+	+	+	+
DL-Malate	+	+	−	−	−	−	−	−	−	−	+	+
L-Arabinose, D-Mannose, N-acetyl-D-glucosamine	−	−	−	−	−	−	−	−	−	−	−	+

[a] Symbols: +, positive; −, negative.

Type strain: Steinle RK1, DSM 11853, EY 4368, GTC 1873.
GenBank accession number (16S rRNA): AF312022, AJ002302.

3. **Ralstonia campinensis** Goris, De Vos, Coenye, Host, Janssens, Brim, Diels, Mergeay, Kersters and Vandamme 2001, 1780[VP]

cam.pin.en′sis. L. adj. *campinensis* pertaining to the ikempen or Campine, the geographical region of northeast Belgium, where the strains were isolated.

The characteristics are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. The cell size is 0.8 × 1.2–1.8 µm. Colonies are round (sometimes with a slightly scalloped margin), smooth, convex, and transparent. The colony diameter is less than 0.5 mm in diameter on TSA agar plate after 24 h incubation at 30°C. Able to grow at 41°C, but not at 4°C. For enzyme activities determined with API Zyme, see Goris et al. (2001). The type strain was isolated from a zinc-decertified area in Lommel, Belgium.

The mol% G + C of the DNA is: 66.6–66.8 (HPLC).
Type strain: WS2, CCUG 44526, EY 4379, GTC 1881, LMG 19282.
GenBank accession number (16S rRNA): AF312020.

4. **Ralstonia eutropha** (Davis, Doudoroff, Stanier and Mandel 1969) Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1996, 625[VP] (Effective publication: Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1995, 903) (*Alcaligenes eutrophus* Davis *in* Davis, Doudoroff, Stanier and Mansel 1969, 386.)

eu.troph′a. Gr. prep. *eu* good, beneficial; Gr. n. *trophus* one who feeds; M.L. n. *eutropha* good nutrition, well nourished.

The characteristics are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. Cells are 0.7 × 1.8–2.6 µm and have peritrichous flagella. Able to grow at 41°C. Resistant to nickel (Schmidt et al., 1991) and to a combination of cobalt and nickel (Tibazarwa et al., 2000). A genetically engineered strain has an enhanced ability to immobilize external Cd^{2+} ions and reduced the toxic effects of heavy metals on the growth of tobacco plants (Valls et al., 2000). Synthesizes short chain length (SCL) (3–5 carbon atoms) PHAs by means of PHA synthase (Song et al., 2000c; Zhang et al., 2000c) and accumulates them as intracellular granules. PHAs are now of commercial value as thermoplastics (Holmes, 1985), and have been extensively studied (Lütke-Eversloh and Steinbuchel, 1999; Grzeszik et al., 2000; Happe et al., 2000; Padilla et al., 2000; Bernhard et al., 2001; Bramer and Steinbuchel, 2001; Drewlo et al., 2001; Schräder et al., 2001; Taguchi et al., 2001; Zarnt et al., 2001). Accumulated PHB is degraded and used for growth and survival when an exogenous carbon source is not available (Handrick et al., 2000). Genetic determinants essential for synthesis of these polymers are linked to the megaplasmid pHG1. Genome of *R. eutropha* strain H16 is composed of two circular chromosomes measuring 4.1 and 2.9 Mb. A physical map of the megaplasmid pHG1 has been given (Schwartz and Fiedrich, 2001). The type strain was isolated from soil with hydrogen gas.

The mol% G + C of the DNA is: 65.5 (HPLC).
Type strain: ATCC 17697, DSM 531, EY 3798, GTC 1874, JCM 11282, LMG 1199.
GenBank accession number (16S rRNA): M32021.

TABLE BXII.β.12. Susceptibilities of type strains of 11 *Ralstonia* species and of the type strain of *Burkholderia cepacia*[a]

Compound (mg/disc)	*R. picketii* EY 4382	*R. basilensis* EY 4368	*R. campinensis* EY 4379	*R. eutropha* EY 3798	*R. gilardii* EY 4363	*R. mannitoli-lytica* EY 4364	*R. metallidurans* EY 4380	*R. oxalatica* EY 4365	*R. paucula* EY 4366	*R. solanacearum* EY 2181	*R. taiwanensis* EY 4381	*Burkholderia cepacia* EY 645
Minocycline (30)	S	S	S	S	S	S	S	S	S	S	S	S
Doxycycline (10)	S	S	S	S	S	S	S	S	IM	S	S	S
Tetracycline (30)	S	S	IM	S	S	S	S	S	IM	S	S	S
Levofloxacin (5)	S	S	S	S	S	S	S	S	S	S	S	Rc
Cefotaxime (30)	S	S	S	S	IM	S	S	S	S	S	S	R
Tosufloxacin (5)	S	S	S	S	S	S	S	S	R	S	S	R
Ciprofloxacin (5)	S	S	S	S	S	S	R	S	R	S	S	R
Ceftazidime (30)	S	S	S	S	R	IM	R	S	S	S	R	R
Norfloxacin (10)	S	S	IM	S	IM	R	R	S	R	S	R	R
Ofloxacin (5), sparfloxacin (5)	S	S	S	S	S	S	S	S	IM	S	S	S
Sulfamethoxazole-trimethoprim (23.75/1.25)	S	S	R	IM	S	R	R	S	R	S	S	R
Imipenem (10)	S	S	R	S	R	R	R	S	R	S	S	R
Carumonam (30)	S	S	R	R	R	R	R	R	R	S	S	R
Cefoperazon (75)	S	R	IM	S	R	IM	S	IM	R	S	S	R
Piperacillin (100)	S	R	R	R	S	R	S	S	R	S	S	R
Cefmetazole (30)	S	R	IM	S	R	R	S	S	R	S	S	R
Trimethoprim (5)	S	R	R	R	R	R	R	S	IM	S	R	S
Flomoxef (30)	IM	R	R	R	R	R	R	IM	R	S	S	R
Cefaclor (30)	S	R	S	R	R	R	R	R	R	S	S	S
Cefazolin (30)	S	R	R	R	R	R	R	R	R	S	R	S
Clarithromycin (15)	R	IM	R	IM	R	R	R	S	R	S	R	R
Polymyxin B (300)	R	R	S	S	S	R	IM	S	S	R	S	R
Moxalactam (30)	R	IM	R	IM	R	R	R	S	R	S	S	R
Meropenem (10)	R	S	R	R	R	R	R	S	R	S	R	R
Roxithromycin (15)	R	S	R	R	R	R	R	IM	R	S	R	R
Erythromycin (15)	R	R	R	R	R	R	R	R	IM	S	IM	R
Penicillin (10), (25)	R	R	R	R	R	R	R	R	R	S	R	R
Ampicillin (10), amoxicillin (25)	R	R	R	R	R	R	S	S	R	S	R	R
AMPCd/CVCe (20/10), panipenem (10), aztreonam (30)	R	R	R	R	R	R	R	S	R	S	R	R
Gentamicin (10), amikacin (30), dibekacin (30)	R	R	S	R	R	R	R	S	R	S	R	R

[a]Symbols: S, susceptible; IM, intermediate; R, resistant.

TABLE BXII.β.13. Assimilation reactions of eleven type strains of *Ralstonia* species and the type strain of *Burkholderia cepacia* by the Biolog GN2 MicroPlate system[a]

Substrate	*Ralstonia pickettii* EY 4382	*Ralstonia basilensis* EY 4368	*Ralstonia campinensis* EY 4379	*Ralstonia eutropha* EY 3798	*Ralstonia gilardii* EY4363	*Ralstonia mannitolilytica* EY 4364	*Ralstonia metallidurans* EY 4380	*Ralstonia oxalatica* EY 4365	*Ralstonia paucula* EY 4366	*Ralstonia solanacearum* EY 2181	*Ralstonia taiwanensis* EY 4381	*Burkholderia cepacia* EY 645
cis-Aconitic acid	+	+	−	+	−	−	+	+	+	+	+	+
Alaninamide	+	−	+	+	+	+	+	+	·	+	−	+
D-Alanine	+	+	−	+	+	−	+	−	−	+	+	+
L-Alanine	+	+	−	+	+	−	+	+	−	+	+	+
L-Alanyl-glycine	+	−	−	−	+	+	+	+	−	+	+	+
γ-Aminobutyric acid, D-glucuronic acid, α-D-glucose	+	−	−	−	−	+	−	−	−	−	−	+
L-Arabinose, glucuronamide	+	+	−	+	+	+	−	+	−	+	+	+
Bromosuccinic acid	+	+	+	+	+	−	+	+	+	+	−	+
Citric acid	+	+	+	+	+	+	+	+	+	+	+	+
Formic acid	+	+	−	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	−	+	−	+	+	−	−	+	+
D-Galactonic acid lactone, D-galactose	+	+	+	−	+	+	+	+	−	−	−	−
D-Galacturonic acid	+	+	+	−	+	+	+	−	−	+	+	+
D-Gluconic acid	+	+	+	−	+	+	+	+	+	+	+	+
Glycerol	+	v	+	+	+	+	+	+	+	+	+	+
Glycogen	−	+	−	−	−	−	−	−	−	−	−	+
Glycyl-L-glutamic acid	+	+	+	−	v	+	+	+	+	+	+	+
L-Histidine, urocanic acid	+	+	−	−	+	+	+	+	+	+	+	+
α-Hydroxybutyric acid	−	+	+	−	+	−	+	+	−	−	−	−
γ-Hydroxybutyric acid	+	+	+	−	+	+	+	+	+	+	+	+
p-Hydroxyphenylacetic acid	−	+	+	−	−	−	+	+	+	−	−	+
Itaconic acid	−	+	+	−	+	+	+	+	+	+	+	+
α-Ketobutyric acid, L-leucine	−	+	+	−	+	+	+	+	+	+	+	+
α-Ketoglutaric acid	−	+	+	−	+	−	−	+	+	+	+	+
α-Ketovaleric acid	−	+	−	−	+	+	+	+	+	+	+	+
Malonic acid	+	+	+	+	+	+	+	+	+	+	+	+
Monomethylsuccinate, propionic acid,	+	+	+	+	+	v	+	+	+	+	+	+
L-Phenylalanine, succinamic acid,	v	+	v	−	+	v	v	−	−	+	−	+
Phenylethylamine	−	+	+	−	−	v	v	−	−	−	−	−
D-Psicose	−	+	+	−	−	−	+	+	+	+	+	+
Quinic acid	+	+	+	−	+	+	+	+	+	+	+	+
D-Saccharic acid	+	+	+	−	+	+	+	+	+	+	+	+
Sebacic acid	+	+	+	−	+	+	+	+	+	+	+	+
D-Serine	+	−	+	−	+	−	+	+	+	+	+	+
L-Serine	+	+	+	+	+	+	+	+	+	+	+	+
L-Threonine	+	+	+	−	+	+	+	+	+	+	+	+
Tween 40, Tween 80	−	+	−	+	−	+	+	+	+	+	+	+
Acetic acid, L-asparagine, L-aspartic acid, L-glutamic acid, β-hydroxybutyric acid, DL-lactic acid, methylpyruvate, L-proline, L-pyroglutamic acid, succinic acid	+	+	+	+	+	+	+	+	+	+	+	+
N-Acetyl-D-galactosamine, cellobiose, α-cyclodextrin, *i*-erythritol, glycyl-L-aspartic acid, inosine, D-raffinose, uridine, α-D-lactose, lactulose, maltose, D-melibiose, β-methyl-D-glucoside, L-rhamnose, D-trehalose, turanose, xylitol	−	−	−	−	−	−	−	−	−	−	−	−
N-Acetyl-D-glucosamine, adonitol, 2-aminoethanol, D-arabitol, 2,3-butanediol, DL-carnitine, dextrin, L-fucose, gentiobiose, glucose-1-phosphate, glucose-6-phosphate, D-glucosaminic acid, DL-α-glycerol phosphate, hydroxy-L-proline, *m*-inositol, D-mannitol, D-mannose, L-ornithine, putrescine, D-sorbitol, sucrose, thymidine	−	−	−	−	−	−	−	−	−	−	−	+

[a] +, positive; − , negative.

TABLE BXII.β.14. Cellular fatty acid composition (% of total) of the type strains of 11 *Ralstonia* species and of *Burkholderia cepacia* [a, b]

Fatty acids, % of total	*R. pickettii* strain EY 4382	*R. basilensis* strain EY 4368	*R. campinensis* strain EY 3797	*R. eutropha* strain EY 3798	*R. gilardii* strain EY 4363	*R. mannitolilytica* strain EY 4364	*R. metallidurans* strain EY 4380	*R. oxalatica* strain EY 4365	*R. paucula* strain EY 4366	*R. solanacearum* strain EY 2181	*R. taiwanenisis* strain EY 4381	*Burkholderia cepacia* strain EY 645
$C_{14:0}$	4	3	5	4	3	3	4	2	4	5	2	5
$C_{15:0}$	tr[c]		tr	tr			tr	tr		1		tr
$C_{16:1\ \omega7c}$	24	14	11	26	12	8	18	12	5	13	23	5
$C_{16:0}$	23	18	30	32	19	20	24	19	18	33	27	36
$C_{17:0}$	1			tr	2		tr	tr		1	tr	tr
$C_{18:1\ \omega9t},$ $C_{18:1\ \omega7c}$	25	13	28	19	18	11	29	14	13	24	25	23
$C_{18:0}$	tr	2	tr	tr	4	3	1	2	3	2	2	5
$C_{20:0}$												tr
$C_{14:0\ 2OH}$	tr	3	2	4			tr	10	2		3	
$C_{16:1\ 2OH}$	4	10	3				2				tr	
$C_{16:0\ 2OH}$			tr		5	9	4		10	1		2
$C_{18:1\ 2OH}$	5	6	4		4		3			8	2	5
$C_{14:0\ 3OH}$	9	31	10	13	31	46	10	39	45	12	11	9
$C_{16:0\ 3OH}$												8
Other	3		6		2		4	tr			4	

[a] N. Fujiwara and T. Naka, unpublished data.

[b] By hydrolysis with HCl-methanol (1:5, v/v) at 100°C for 3 h.

[c] tr, <1%.

5. **Ralstonia gilardii** Coenye, Falsen, Vancanneyt, Hoste, Govan, Kersters and Vandamme 1999a, 412[VP]

gi.lar' di.i. M.L. gen. n. *gilardii* named after G.L. Gilardi, an American microbiologist who contributed much to our knowledge of *Alcaligenes* species.

The characteristics are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. The cell size is 0.7 × 1.8–2.6 µm. Motile by means of a single polar flagellum. Able to grow at 41°C. Growth occurs in the presence of 5.0% NaCl.

The mol% G + C of the DNA is: 68–69 (T_m).

Type strain: ATCC 700815, EY 4363, GTC 1875, JCM 11283, LMG 5886.

GenBank accession number (16S rRNA): AF076645.

6. **Ralstonia mannitolilytica** De Baere, Steyaert, Wauters, De Vos, Goris, Coenye, Suyama, Verschraegen and Vaneechoutte 2001, 556[VP]

man.ni.to.li.ly' ti.ca. N.L. adj. *mannitolilytica* cleaving mannitol.

The species accommodates strains previously known as the *Ralstonia pickettii* biovar 3/ *'thomasii'* strains and at least some of the strains known as *"Pseudomonas thomasii"* Phillips et al. 1972. The characteristics are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. Motile by means of single polar flagellum, with the only exception being the type strain. Able to grow at 41°C. For enzymatic activity, see the results by means of API ZYM (De Baere et al., 2001). The type strain was isolated from blood of patient at St. Thomas' Hospital, London, UK, in 1971 (Phillips et al., 1972).

The mol% G + C of the DNA is: 66.2 (HPLC).

Type strain: LMG 6866, EY 4364, GTC 1876, JCM 11284, NCIB 10805.

GenBank accession number (16S rRNA): AJ270258.

7. **Ralstonia metallidurans** Goris, De Vos, Coenye, Host, Janssens, Brim, Diels, Mergeay, Kersters and Vandamme 2001, 1780[VP]

me.tal.li.du' rans. L. n. *metallum* metal; L. pres. part. *durans* enduring; N.L. part. adj. *metallidurans* enduring metal, to indicate that these strains are able to survive high heavy-metal concentrations.

The characteristics of the type strain are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. Cells are short motile rods 0.8 × 1.2–2.2 µm. Colonies are round (sometimes with a slightly scalloped margin), smooth, slightly convex, and beige colored when fully grown. Unable to grow either at 4° or 41°C. For enzyme activities, see Goris et al. (2001). The type strain was isolated from wastewater from a zinc factory at Liege, Belgium.

The mol% G + C of the DNA is: 63.7 (HPLC).

Type strain: DSM 2389, EY 4380, GTC 1882, LMG 1195.

GenBank accession number (16S rRNA): Y10824.

8. **Ralstonia oxalatica** (ex Khambata and Bhat 1953) Sahin, Isik, Tamer and Goodfellow 2000b, 1953[VP] (Effective publication: Sahin, Isik, Tamer and Goodfellow 2000a, 207.)

o.xa.la' ti.ca. M.L. fem. adj. *oxalatica* pertaining to oxalate.

The characteristics of the type strain are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. Motile by means of one polar or subpolar flagellum. Cell size is 0.3–0.4 × 0.9–1.5 µm. Able to grow on ordinary peptone media without supplementing with oxalate. Unable to grow autotrophically with hydrogen. The type strain was isolated from the alimentary tract of an Indian earthworm.

The mol% G + C of the DNA is: 68 (T_m) or 67 (Bd).

Type strain: ATCC 11883, DSM 1105, EY 4365, JCM 11285, NCIB 8642, LMG 2235.

GenBank accession number (16S rRNA): AF155567.

9. **Ralstonia paucula** Vandamme, Goris, Coenye, Hoste, Jenssens, Kersters, De Vos and Falsen 1999, 668[VP]

pau' cu.la. L. adj. *pauculus* rare, very few, to indicate that these strains only sporadically cause human infections.

The characteristics of the type strain are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. Cell size is 0.8 × 1.2–2.0 μm. Motile by means of peritrichous flagella. Does not denitrify. These organisms were previously placed in CDC group Ivc-2. Isolated from a variety of human clinical sources (Vandamme et al., 1999) as well as environmental sources such as pool water, groundwater, and bottled mineral water. Differentiated from other species by peritrichous flagellation, ability to form acid oxidatively from L-arabinose, and absence of cystine arylamidase, phosphoamidase, and lipase C_{14} activity. The type strain was isolated from a human respiratory tract in USA.

The mol% G + C of the DNA is: 65–67 (T_m).

Type strain: CCUG 12507, CDC E6793, EY 4366, GTC 1878, JCM 11286, LMG 3244.

GenBank accession number (16S rRNA): AF085226.

10. **Ralstonia solanacearum** (Smith 1896) Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1996, 625[VP] (Effective publication: Yabuuchi, Kosako, Yano, Hotta and Nishiuchi 1995, 903.) (*Pseudomonas solanacearum* (Smith 1896) Smith 1914, 178; *Bacillus solanacearum* Smith 1896, 10.)

so.la.na.ce.a′ rum. M.L. fem. pl. n. *Solanaceae* the nightshade family; M.L. fem. pl. gen. n. *solanacearum* of the *Solanaceae*.

The characteristics of the type strain are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. Nonmotile. Pathogenic for tomato, potato, tobacco, banana, and peanut by the production of a copious amount of extracellular polysaccharide, which occludes vessels in the stem, inhibits water flow, and causes death of plant by wilt disease. Recently the nucleotide full sequence of the chromosome and megaplasmid were determined in order to elucidate and manipulate genes related to the virulence of the organism. The type strain was isolated from tomato.

The mol% G + C of the DNA is: 66.6 (HPLC).

Type strain: ATCC 11696, DSM 9544, EY 2181, GTC 1879, JCM 10489, LMG 2299, NCPPB 325.

GenBank accession number (16S rRNA): X67036.

11. **Ralstonia taiwanensis** Chen, Laevens, Lee, Coenye, De Vos, Mergeay and Vandamme 2001, 1734[VP]

tai.wan.en′ sis. N.L. fem. adj. *taiwanensis* pertaining to Taiwan, where the root nodule strains were isolated.

The characteristics of the type strain are as described for the genus and listed in Tables BXII.β.10, BXII.β.11, BXII.β.12, BXII.β.13, and BXII.β.14. Motile; type of flagellation unknown. Cell size, 0.5–0.7 × 0.8–2.0 μm. Growth is observed at 28°, 30°, and 37°C. Nitrate is reduced. Esculin is hydrolyzed.; gelatin and Tween 80 are not. Susceptible to tetracyclines and quinolines; resistant to β-lactams and aminoglycosides. Urease, β-galactosidase, and DNase are negative. Indole is not produced. Autotrophic growth does not occur. The type strain was isolated from root nodules of *Mimosa pudica*.

The mol% G + C of the DNA is: 67.3 (HPLC).

Type strain: R1, CCUG 44338, EY 4381, GTC 1883, LMG 19424.

GenBank accession number (16S rRNA): AF300324.

Genus VIII. *Thermothrix* Caldwell, Caldwell and Laycock 1981, 217[VP] (Effective publication: Caldwell, Caldwell and Laycock 1976, 1515)

ANNA-LOUISE REYSENBACH, PAULA AGUIAR AND DOUGLAS E. CALDWELL

Ther′ mo.thrix. Gr. adj. *thermos* hot; Gr. n. *thrix* hair; N.L. fem. n. *Thermothrix* hot hair.

Rod-shaped cells, usually 0.5–1.0 × 3–5 μm. **Filamentous cells are produced** under unfavorable growth conditions such as when grown at temperatures near the maximum temperature, at extreme pH values, or when oxygen limits growth. **Motile by means of a single polar flagellum**. Gram negative. No spores are produced. **Growth occurs between 63–86°C and pH 6–8.5. Facultatively or obligately chemolithoautotrophic aerobic**. Oxygen or nitrate may be used as the electron acceptor. Electron donors can be organic compounds or inorganic sulfur compounds such as thiosulfate, sulfur, hydrogen sulfide, and tetrathionate.

The mol% G + C of the DNA is: 39.7.

Type species: **Thermothrix thiopara** Caldwell, Caldwell and Laycock 1981, 217 (Effective publication: Caldwell, Caldwell and Laycock 1976, 1515.)

FURTHER DESCRIPTIVE INFORMATION

Elemental sulfur is often deposited extracellularly as spherical granules (Figs. BXII.β.8 and BXII.β.9), although *T. azorensis* accumulates sulfur intracellularly when the oxidation of thiosulfate is incomplete and the pH does not drop below pH 7.0. Respired substrates may include glucose, sulfide, and thiosulfate. When thiosulfate is oxidized to sulfate, elemental sulfur, sulfite, and polythionate accumulate as intermediates in batch culture (Brannan and Caldwell, 1980, 1983).

This genus was found within the sulfide-oxygen interfaces of neutral pH geothermal springs, including Mammoth Hot Springs (Yellowstone National Park, Wyoming, USA), Jemez Hot Springs (Jemez Springs, New Mexico, USA), and Furnas (Sao Miguel Island, Azores).

The isolates of *Thermothrix* were obtained from thermal springs characterized by "sulfur turf" mats. These mats generally consist of a mixture of filamentous and rod-shaped cells, intertwined to form "streamers" up to 10 cm in length. Although many *in situ* studies have been conducted on these mats, recent molecular phylogenetic assessments of the Mammoth Hot Springs, the Jemez Spring, and the Furnas "streamers" revealed the dominance of *Aquificales*-like 16S rRNA sequences and no beta-proteobacterial sequences (A.L. Reysenbach, P. Aguiar, and T. Kieft, unpublished data). Additionally, fluorescent *in situ* 16S rRNA probes, specific for the order *Aquificales*—in the class *Aquificae*, phylum *Aquificae*—confirmed that this group of organisms forms the primary matrix of these mats (Fig. BXII.β.10). Based on this new molecular evidence, it is therefore unclear how significant the role of *Thermothrix*—in the class *Betaproteobacteria*, phylum *Proteobacteria*—is in the formation of the mats.

ENRICHMENT AND ISOLATION PROCEDURES

Representatives of the genus are found within the sulfide-oxygen interface of terrestrial hot springs (40–80°C). Under these con-
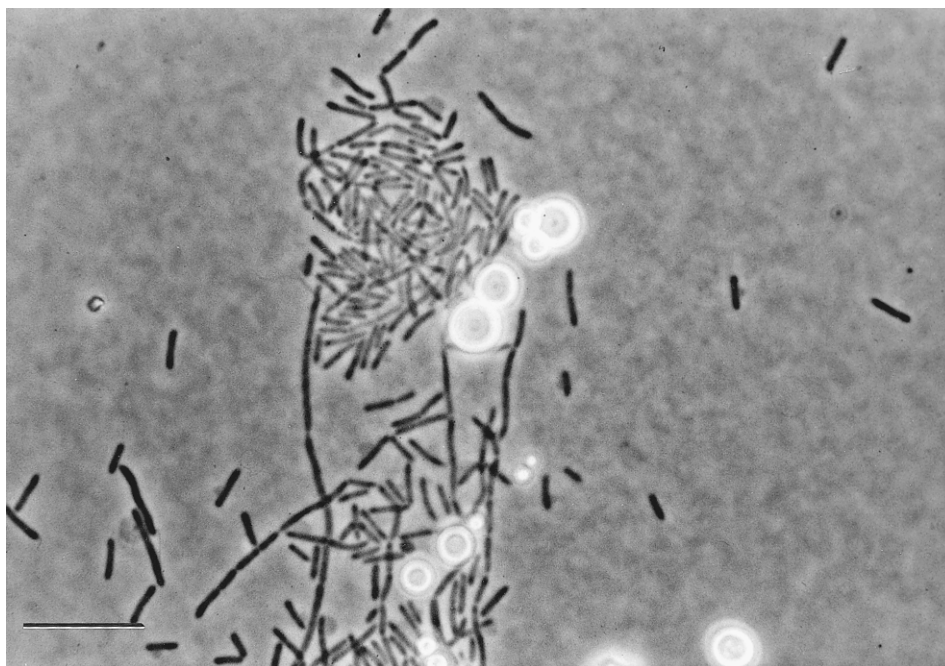
FIGURE BXII.β.8. *T. thiopara* during oxidation of thiosulfate, with extracellular deposition of elemental sulfur (spherical granules) shown. The culture was highly aerated, thus producing rod-shaped cells and cell chains but no cell filaments. Bar = 10 μm.
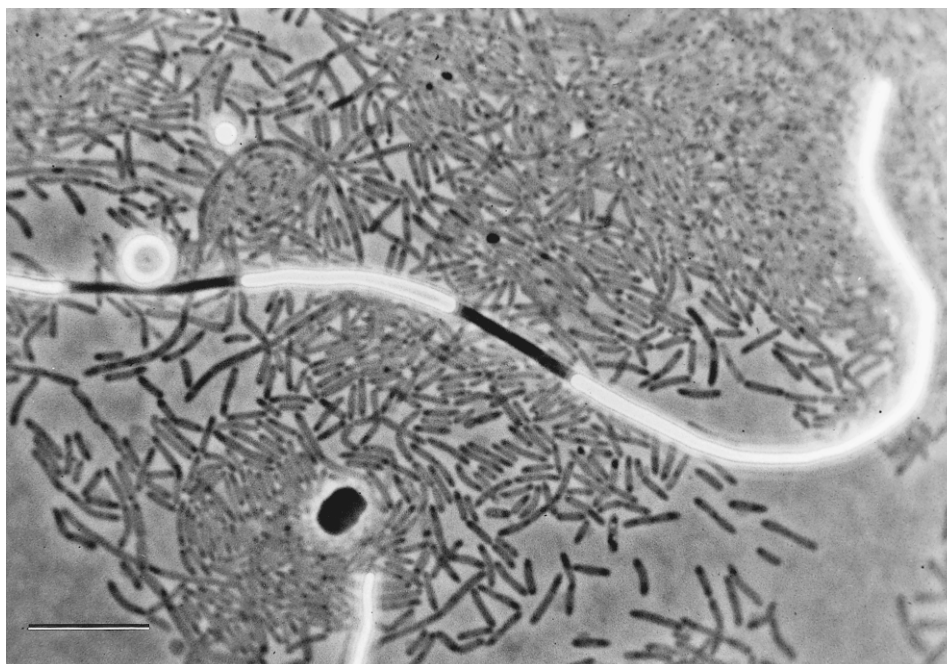

FIGURE BXII.β.9. Deposition of elemental sulfur in a smooth refractile layer partially surrounding a filament of *T. thiopara* (*center*) during the transition from nitrate broth to a synthetic medium with thiosulfate as sole energy source. Phase-contrast micrograph. Bar = 10 μm.

ditions, sulfide serves as the electron donor and oxygen as the electron acceptor. Consequently, a synthetic medium with either sulfide or thiosulfate as the sole energy source can be used for initial cultivation. The following medium has been used (g/l): $Na_2S_2O_3 \cdot 5H_2O$, 3.0; $NaHCO_3$, 2.0; NH_4Cl, 1.0; KNO_3, 2.0; $MgSO_4 \cdot 7H_2O$, 0.5; KH_2PO_4, 2.0; and $FeSO_4 \cdot 7H_2O$-EDTA, 0.02;

plus trace elements (pH 6.8) (Brannan and Caldwell, 1980). Care should be taken to avoid the loss of bicarbonate as carbon dioxide if the medium is autoclaved and when the medium is stored and incubated. The enrichments are incubated at 73°C and should be transferred during logarithmic phase (reached after about 24 h), or stored in log phase of growth at 4°C, as cell death may
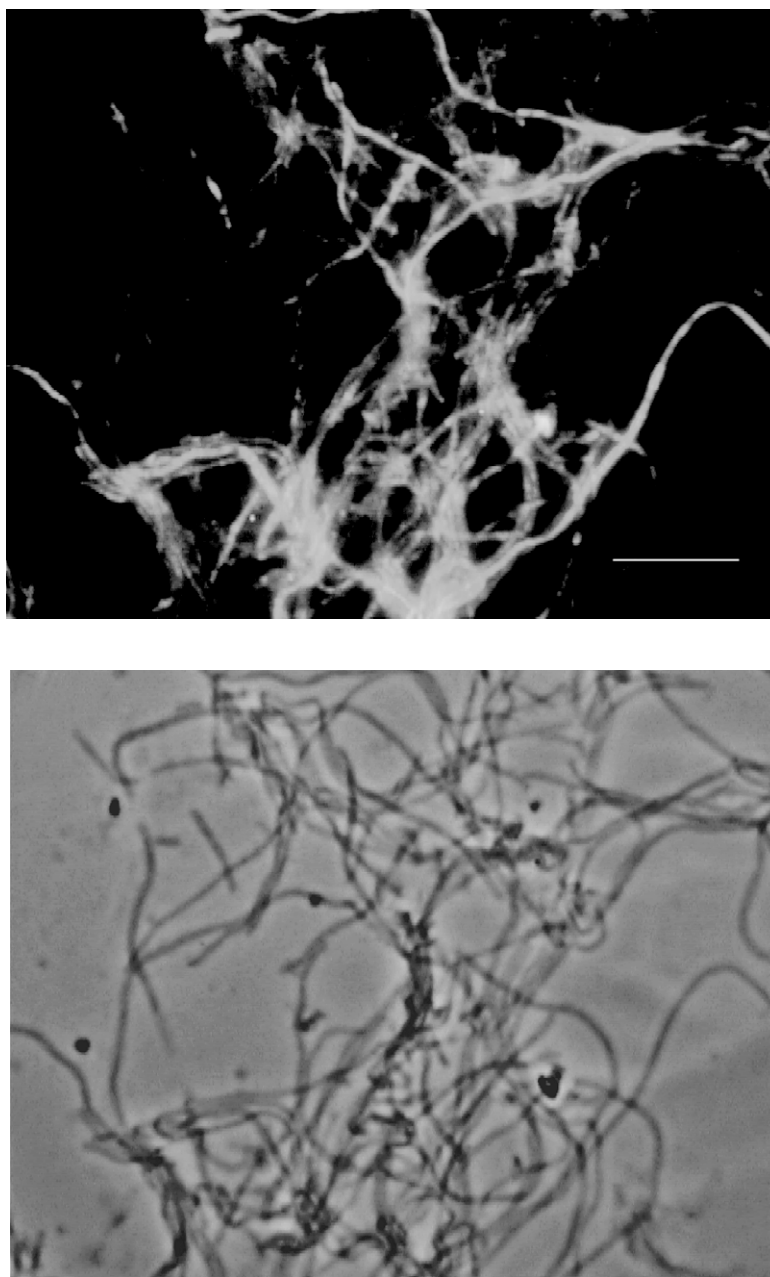
FIGURE BXII.β.10. *Upper panel*, streamers collected from Jemez Springs, New Mexico, probed with a fluorescein-labeled oligonucleotide 16S rDNA probe specific for the 16S rRNA from the *Aquificales* (Harmsen et al., 1997). *Bottom panel*, phase contrast micrograph of same frame. Bar = 10 µm.

occur within 4 h of reaching the stationary growth phase. Because of the low solubility of the oxygen at 73°C (approximately 3 mg/l), the organisms should be cultivated in shake flasks with a large headspace to provide sufficient aeration. Isolated colonies may be obtained on agar or Gelrite; however, recovery is poor. After initial isolation, some species of *Thermothrix* may be adapted, with difficulty, to grow on an organic medium such as a nutrient (aerobic) or nitrate (anaerobic) broth.

MAINTENANCE PROCEDURES

Cells may be frozen in a 15% glycerol (v/v) and stored at −80°C. Cultures of *T. thiopara* adapted to grow on nitrate broth (Difco)

were more easily maintained than were autotrophically grown cells.

DIFFERENTIATION OF THE GENUS *THERMOTHRIX* FROM OTHER GENERA

The primary basis for discrimination of *Thermothrix* species from thermophilic *Thiobacillus* species is the production of filaments when the concentration of the electron acceptor limits the rate of growth. Cells obtained during the initial isolation are usually motile and rod shaped. Induction of the filamentous growth form requires continuous cultivation under oxygen-limited con-

ditions. The oxygen tension should be maintained at 1 mg/l or less.

FURTHER READING

Brannan, D.K. and D.E. Caldwell. 1980. *Thermothrix thiopara*: growth and metabolism of a newly isolated thermophile capable of oxidizing sul-

fur and sulfur compounds. Appl. Environ. Microbiol. *40*: 211–216.
Odintsova, E.V., H.W. Jannasch, J.A. Mamone and T.A. Langworthy. 1996. *Thermothrix azorensis* sp. nov., an obligately chemolithoautotrophic, sulfur-oxidizing, thermophilic bacterium. Int. J. Syst. Bacteriol. *46*: 422–428.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *THERMOTHRIX*

Only two species are described: *Thermothrix thiopara* and *Thermothrix azorensis*. These have been distinguished by their 16S rRNA structure, temperature growth optima, and whether they are obligately or facultatively chemolithoautotrophs and obligately or facultatively aerobic.

List of species of the genus Thermothrix

1. **Thermothrix thiopara** Caldwell, Caldwell and Laycock 1981, 217[VP] (Effective publication: Caldwell, Caldwell and Laycock 1976, 1515.)

   thi.o′par.a. Gr. n. *thios* sulfur; L. v. *paro* produce; M.L. adj. *thiopara* sulfur-depositing.

   See the description of the genus for most features of the species. The cardinal temperatures are 60°, 73°, and 80°C (minimum, optimum, and maximum) for cells grown by using either inorganic or organic media (Caldwell et al., 1976; Brannan and Caldwell, 1982). The generation time at 73°C is approximately 2 h on either medium. When reduced sulfur compounds are used as the electron donor, elemental sulfur is frequently deposited as extracellular granules but is not deposited intracellularly (Fig. BXII.β.8). In batch culture, elemental sulfur, sulfite, and polythionates accumulate as transient intermediates but are subsequently oxidized to sulfuric acid. In continuous culture, elemental sulfur is not deposited during steady-state growth but is deposited during the transition from low to high dilution rates. During transfer from nitrate broth to autotrophic media containing thiosulfate, cell filaments often form and become encased in a coating of elemental sulfur (Fig. BXII.β.9).

   Denitrification occurs only heterotrophically (Brannan and Caldwell, 1982). During denitrification, N₂ is the primary end product. Nitrite accumulates as a transient intermediate.

   The mol% G + C of the DNA is: 39.7 (T_m).
   Type strain: ATCC 29244.
   GenBank accession number (16S rRNA): U61284.
   Additional Remarks: The original strain is no longer available from the ATCC.

2. **Thermothrix azorensis** Odintsova, Jannasch, Mamone and Langworthy 1996, 426[VP]

   a.zo′ren.sis. L. fem. adj. *azorensis* from the Azores.

   Long, thin rods (2–5 × 0.3–0.5 μm) often in pairs. Filaments up to 70 μm long are produced under unfavorable growth conditions. Gram-negative nonsporulating cells, some contain sulfur inclusions. Strictly aerobic and obligately chemolithoautotrophic, using reduced sulfur compounds (hydrogen sulfide, tetrathionate, elemental sulfur, and thiosulfate) as electron donors and carbon dioxide as the carbon source. During the lag phase of growth, elemental sulfur oxidation is preceded by sulfide production. Grows best between 76° and 78°C, pH 7.0–7.5. Temperature and pH ranges for growth are 60–87°C and pH 6.0–8.5, respectively. Lipids include C₁₄ to C₂₂ fatty acids (95% C₁₆ and C₁₈). No ribulose-1,5-bisphosphate carboxylase/oxygenase activity is present.

   Isolated from a hot spring located at Furnas, Sao Miguel, the Azores, Portugal.
   The mol% G + C of the DNA is: 39.7 (T_m).
   Type strain: TM, ATCC 51754.
   GenBank accession number (16S rRNA): U59127.

Family II. **Oxalobacteraceae** *fam. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Ox.al.o.bac.ter.a′ce.ae. M.L. masc. n. *Oxalobacter* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Oxalobacteraceae* the *Oxalobacter* family.

The family *Oxalobacteraceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the family contains the genera *Oxalobacter* (type genus), *Duganella*, *Herbaspirillum*, *Janthinobacterium*, *Massilia*, *Oxalicibacterium*, and *Telluria*. *Oxalicibacterium* was proposed after the cut-off date for inclusion in this volume (June 30, 2001) and is not described here (see Tamer et al. (2002)).

Family is metabolically diverse and includes strict anaerobes, strict aerobes, and nitrogen-fixing organisms.

Type genus: **Oxalobacter** Allison, Dawson, Mayberry and Foss 1985b, 375[VP] (Effective publication: Allison, Dawson, Mayberry and Foss 1985a, 6.)

Genus I. **Oxalobacter** Allison, Dawson, Mayberry and Foss 1985b, 375[VP] (Effective publication: Allison, Dawson, Mayberry and Foss 1985a, 6)

MILTON J. ALLISON, BARBARA J. MACGREGOR, RICHARD SHARP AND DAVID A. STAHL

Ox.al.o.bac' ter. Gr. n. *oxal* pertaining to oxalate; M.L. n. *bacter* the masculine form of the Gr. neut. n. *bactrum* a rod; M.L. masc. n. *Oxalobacter* an oxalate rod.

Straight or curved to vibrioid, Gram-negative, nonsporeforming rods 0.4–0.6 × 1.0–2.5 µm in length. Flagella may be present or absent. **Strictly anaerobic.** Chemoorganotroph. **Oxalate is used as the major carbon and energy source. Oxamate may also be used, but neither carbohydrates nor any of a wide variety of other compounds will replace oxalate as the growth substrate.** Acetate is assimilated for cell synthesis and is required by some, and perhaps by all, strains. Oxalate utilization is accompanied by alkalization of the medium, and formate is produced in approximately equimolar proportions to the amount of oxalate metabolized. Strains have been isolated from the rumens of cattle and sheep, from cecal and fecal samples from humans, guinea pigs, swine, domestic and wild rats, and from freshwater lake and marine sediments. It is probable that these bacteria colonize many other anaerobic habitats. *Oxalobacter* is currently classified in the class *Betaproteobacteria*, the order *Burkholderiales*, and the family *Oxalobacteraceae*.

The mol% G + C of the DNA is: 48–52.

Type species: **Oxalobacter formigenes** Allison, Dawson, Mayberry and Foss 1985b, 375 (Effective publication: Allison, Dawson, Mayberry and Foss 1985a, 6.)

FURTHER DESCRIPTIVE INFORMATION

Cultural and growth characteristics In anaerobic roll-tube cultures, colonies on the agar surface are colorless, transparent, and droplet-like, while subsurface colonies are colorless or white and are lens-shaped or globular. Colonies of anaerobic oxalate-degraders can be detected based on production of clear zones around colonies in media containing appropriate amounts of calcium oxalate.

Growth occurs under anaerobic conditions, under a gas phase of CO_2, in a defined carbonate–bicarbonate buffered medium that contains minerals, oxalate, and acetate, but growth is better with yeast extract in the medium. Specific growth rates (µ) may be as high as 0.3 h^{-1}. Isolations have been obtained on media containing 20–50 mM oxalate. Some freshly isolated strains are inhibited by 30 mM oxalate; laboratory adapted strains, however, grow well in media containing oxalate at concentrations of 100 mM or more. Acetate is required for growth of some, and perhaps all, intestinal strains, as well as by strains isolated from sediments. The temperature range for growth is 14–45°C, with gastrointestinal strains exhibiting a higher optimal temperature (about 37°C) than lake sediment strains.

Metabolism and metabolic pathways Oxalate serves as both the energy yielding substrate and the major source of carbon for growth. The products from oxalate metabolism are CO_2 and formate, with approximately 1 mol of each produced per mole of oxalate degraded. Approximately 1–1.7 g (dry wt.) of cells are produced per mole of oxalate degraded. Energy generation is centered around the development of a proton motive force through the electrogenic exchange of oxalate (in) and formate (out) across the cell membrane and the consumption of a proton inside the cell when the CoA-ester of oxalate is decarboxylated by oxalyl CoA-decarboxylase (Anantharam et al., 1989; Kuhner et al., 1996). An antiporter protein (OxlT) facilitates the oxalate–

formate exchange (Ruan et al., 1992). Oxalate serves as a major source of cell carbon, but acetate and carbon dioxide are also used for biosynthesis (Cornick and Allison, 1996b). In tests with benzyl viologen as the electron acceptor, formate dehydrogenase activity is detected in both *O. formigenes* and *O. vibrioformis* (Dehning and Schink, 1989b; Cornick and Allison, 1996a). However, since neither NAD nor NADP are reduced in such tests and since formate accumulation is roughly equivalent to the amount of oxalate degraded, no role for formate dehydrogenase in the energy metabolism of *Oxalobacter* is expected. Neither nitrate nor sulfate are reduced, indole is not formed, and cytochromes are not present.

Pathogenicity There is no evidence that these bacteria, which are normal inhabitants of the gastrointestinal tract, are pathogenic. Large quantities of cells (10^{10}), representing strains isolated from sheep rumen, pig cecum, human feces, guinea pig cecum, and wild rats, have been fed to laboratory rats without ill effects (Daniel et al., 1987a).

Ecology Oxalate, a product of both plant and animal metabolism, is widely distributed in many ecosystems. Metabolism of oxalate by *O. formigenes* in freshwater sediments and gut habitats occurs under anaerobic conditions and at near neutral pH. Metabolism is accompanied by alkalization, since 1 mol of protons is consumed per mole of oxalate metabolized. Formate produced from oxalate has been used for methanogenesis in enrichment cultures of rumen microbes (Dawson et al., 1980a). Normal diets of humans and animals usually contain enough oxalate to maintain intestinal populations of *O. formigenes*. Increased concentrations of these bacteria in the intestine are selected for when diets with elevated concentrations of oxalate are fed to animals. This selection leads to increased rates of degradation of otherwise potentially toxic amounts of oxalate, a phenomenon that has survival value for the host animal (Shirley and Schmidt-Nielsen, 1967; Allison and Cook, 1981). The absence of *O. formigenes* in the intestinal tracts of some humans appears to be correlated with increased absorption of dietary oxalate, leading to increased risk for kidney stones and other oxalate-related disease (Sidhu et al., 1998).

Antibiotic sensitivity Strains HC1 and OxK are both sensitive to chloramphenicol, colistin, doxycycline, erythromycin, polymyxin B, rifampin, and tetracycline. Strain OxK is also sensitive to cefuroxime. Both strains are resistant to 22 other antibiotics that have been tested. Similar patterns have been observed with *O. formigenes* strains from rats (Daniel et al., 1987b) and with the type strain, OxB (Dawson, 1979).

ENRICHMENT AND ISOLATION PROCEDURES

Anaerobic media with oxalate as the main, or only, energy source are used for enrichment cultures from either rumen samples (Dawson et al., 1980a, b) or freshwater lake sediments (Smith et al., 1985; Dehning and Schink, 1989b). Carbonate-buffered media, heated to remove dissolved oxygen and reduced with cysteine and/or sulfide, are used for enrichment cultures. Media for enrichment from sediments also contain acetate and either yeast

extract or a mixture of vitamins, while media for enrichment from the rumen contain rumen fluid.

Colonies of oxalate-degrading bacteria are identified based on the formation of clear zones around the colonies in media that are somewhat opaque due to the presence of calcium oxalate. Daniel et al. (1989) have compared variations in levels of calcium, yeast extract, and rumen fluid on culture counts of oxalate-degrading rumen bacteria, as detected by clear zones around colonies in roll-tube cultures. This method has been used for isolations of *O. formigenes* strains without prior enrichment steps and for enumeration of these bacteria from gastrointestinal samples from man and animals (Allison et al., 1985a).

MAINTENANCE PROCEDURES

Viable cultures may be recovered from cells stored at $-80°C$ for several years or from freeze-dried cells stored at 0–4°C.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Successful detection of *O. formigenes* in fecal samples can be accomplished using PCR amplification of selected sequences of the *oxc* gene (Sidhu et al., 1997a, 1999). Tests can be successfully conducted with fecal samples that have not been cultured or with enrichment cultures in a medium containing 10–30 mM oxalate. The loss of oxalate from such media is usually nearly complete, and a simple test for this loss can be made based on precipitate formation when a calcium chloride solution is added (Sidhu et al., 1997b).

DIFFERENTIATION OF THE GENUS *OXALOBACTER* FROM OTHER GENERA

Organisms of the genus *Oxalobacter* are the only known Gram-negative, anaerobic, oxalate degraders that are extreme specialists, to the extent that oxalate and/or the closely related compound oxamate are obligate requirements for growth. Oxalate serves as the major carbon and energy source, but a small amount of acetate may also be required.

Selected regions of the *oxc* gene (encoding oxalyl-CoA decarboxylase) and the *frc* gene (encoding formyl-CoA transferase) have been used to construct oligonucleotide probes able to distinguish *O. formigenes* DNA from DNA of any of a known group of strains of gastrointestinal bacteria. Further evidence for the specificity of these probes was obtained when it was found that they fail to hybridize or to amplify PCR products from whole fecal DNA isolated from fresh stool samples from an individual known not to be colonized with oxalate-degrading bacteria (Sidhu et al., 1997a).

TAXONOMIC COMMENTS

Similarity values based on 16S rRNA sequences (1440 nucleotides) that allow *Oxalobacter* (represented by strain OxCR of *O. formigenes*) to be compared with other selected species are given in Table BXII.β.15. The corresponding phylogenetic tree (Fig. BXII.β.11) illustrates that strains of *Oxalobacter* within the class *Betaproteobacteria* share a specific relationship with the other genera currently in the *Oxalobacteraceae* family, *Telluria*, *Janthinobacterium*, and *Duganella*.

List of species of the genus Oxalobacter

1. **Oxalobacter formigenes** Allison, Dawson, Mayberry and Foss 1985b, 375[VP] (Effective publication: Allison, Dawson, Mayberry and Foss 1985a, 6.)

form.i.ge' nes. M.L. n. *acidum formicum* formic acid; Gr. v. *gennaio* produce; M.L. adj. *formigenes* formic acid producing.

The characteristics are as described for the genus with

TABLE BXII.β.15. 16S rRNA sequence similarities

	Oxalobacter formigenes OxCR	*Alcaligenes eutrophus* [a]	*Burkholderia gladioli* pathovar *gladioli*	*Burkholderia solanacearum* [a]	*Escherichia coli*	*Iodobacter fluviatilis*	*Neisseria elongata*	*Pseudomonas pickettii* [a]	*Pseudomonas solanacearum* [a]	*Zoogloea ramigera* 4	*Zoogloea ramigera* 5
Oxalobacter formigenes OxCR	—										
Alcaligenes eutrophus	88.8	—									
Burkholderia gladioli pathovar *gladioli*	81.9	88.6	—								
Burkholderia solanacearum	88.4	96.1	91.4	—							
Escherichia coli	78.4	81.2	81.9	81.1	—						
Iodobacter fluviatilis	86.1	88.6	90.4	89.3	82.9	—					
Neisseria elongata	85.2	88.6	88.6	87.2	81.9	89.4	—				
Pseudomonas pickettii	91.2	96.1	89.2	97.8	81.2	89.2	89.2	—			
Pseudomonas solanacearum	88.2	95.8	89.6	99.2	81.1	89.6	89.6	97.8	—		
Zoogloea ramigera 4	88.4	88.5	91.8	90.1	81.1	88.6	88.6	90.4	91.2	—	
Zoogloea ramigera 5	89.3	90.3	90.5	90.1	80.2	88.1	86.4	89.4	90	98.2	—

[a]*Alcaligenes eutrophus* = *Ralstonia eutropha*; *Burkholderia solanacearum* = *Pseudomonas solanacearum* = *Ralstonia solanacearum*; *Pseudomonas pickettii* = *Ralstonia pickettii*.
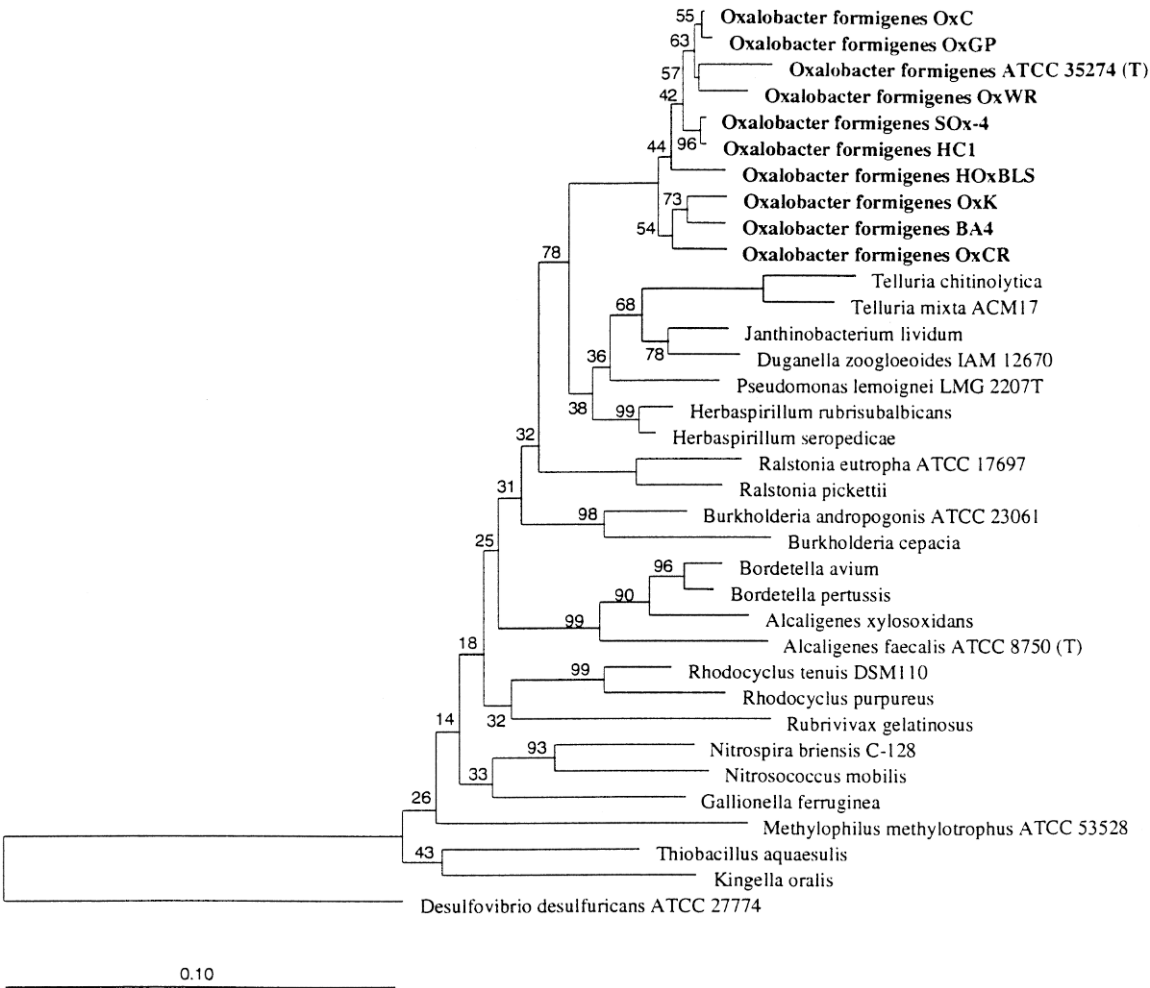
FIGURE BXII.β.11. Phylogenetic tree of *Oxalobacter formigenes* and other representatives of the *Betaproteobacteria* based on 16S rRNA sequence comparisons. The tree was constructed using the neighbor-joining method of Saitou and Nei (1987). Confidence limits were calculated by the bootstrap method using 100 samplings of the data set. Bar = 0.10 estimated substitutions per nucleotide position. *Desulfovibrio desulfuricans*, a member of the *Deltaproteobacteria*, was used to root the tree.

the following additional features. Rods 0.4–0.6 × 1.2–2.5 μm, with rounded ends, often curved, occurring singly, in pairs, or occasionally in chains, with curved cells leading to a spiral appearance. Flagella are not present.

High levels of 16S rRNA sequence similarity (96.6–99.8%) have been found for a group of strains of *O. formigenes* that includes both gastrointestinal and lake sediment strains (Table BXII.β.16).

The major total cellular fatty acids of representative

strains of *O. formigenes* are given in Table BXII.β.17. When strains are arranged based on descending values for quantities of 19-carbon cyclopropane ($C_{19:0 \ cyclo}$) fatty acids, a separation into two groups is evident. When these fatty acid profiles are compared using a cluster analysis program (Sasser, 1990b), the same grouping is supported (Fig. BXII.β.12). Assignment of strains into Groups I and II based on cellular fatty acid profiles generally agrees with grouping based on 16S rRNA sequences (Fig. BXII.β.11), except for

TABLE BXII.β.16. 16S rRNA sequence similarity matrix

Oxalobacter formigenes strains	OxCR	HOxBLS	BA4	Ox-K	OxWR	Ox-B	OxGP	OxC	HC1	SOx4
OxCR	—	—	—	—	—	—	—	—	—	—
HOxBLS	96.9	—	—	—	—	—	—	—	—	—
BA4	97.7	97.7	—	—	—	—	—	—	—	—
Ox-K	96.9	97.1	98.2	—	—	—	—	—	—	—
OxWR	96.6	97.1	96.8	96.4	—	—	—	—	—	—
Ox-B	96.9	97.4	97.3	97	98.2	—	—	—	—	—
OxGP	97.4	97.7	97.5	97	98.4	98.7	—	—	—	—
OxC	97.4	97.7	97.7	97.1	98.6	99	99.5	—	—	—
HC1	97.1	97.9	98.2	96.9	97.9	98.2	98.4	98.7	—	—
SOx4	97.4	98.2	98.3	97	98.1	98.3	98.6	98.9	99.8	—

TABLE BXII.β.17. Major cellular fatty acids in different strains of *Oxalobacter formigenes*[a]

Fatty acid	$C_{14:0}$	$C_{16:0}$	$C_{17:0 \text{ cyclo}}$	$C_{18:1}$	$C_{18:0}$	$C_{19:0 \text{ cyclo}}$
Oxalobacter formigenes strains:						
GROUP I						
PoxC	1.41	39.03	31.14	1.97	1.20	12.54
Sox-6	1.05	40.19	29.74	2.43	0.78	14.88
OxDB12	0.87	35.61	28.76	5.76	0.93	16.05
OxWR	0.87	40.04	29.63	2.80	0.52	16.07
HC3	0.99	36.89	31.28	2.51	0.53	16.35
SOx4	0.98	40.35	29.72	1.35	0.68	16.45
HC-1	1.02	36.45	31.57	2.29	0.40	16.67
OxCC13	1.00	36.72	29.59	3.16	nr[b]	16.85
OxB	0.77	40.70	27.38	1.06	1.18	19.10
OxHM18	1.16	36.08	30.45	0.67	nr	20.06
GROUP II						
HOxBLS	3.50	35.01	12.90	8.45	1.11	31.63
HOxRW	3.56	35.95	12.53	7.84	1.58	31.80
BA1	2.97	32.46	5.69	11.03	2.71	37.61
BA6	3.85	34.04	6.16	7.05	1.64	39.24
OxK	3.21	28.54	2.91	12.63	2.86	42.83
OxGP	2.82	23.95	1.31	14.27	1.47	49.56
89-112	2.56	16.87	0.55	16.96	2.77	53.42

[a]Fatty acids separated as methyl esters by gas chromatography and identified as described by Sasser (1990a).

[b]nr, not reported.
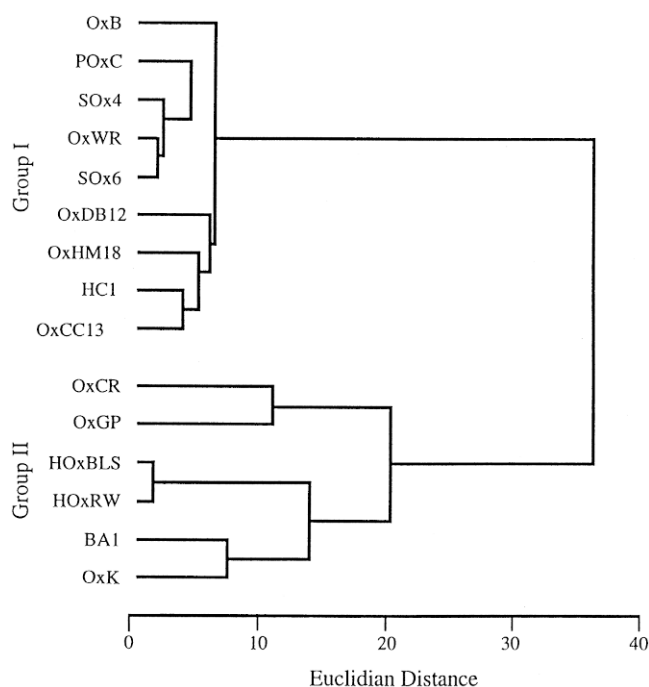
FIGURE BXII.β.12. Computer-generated dendogram (Sasser, 1990b) based on fatty acid profiles of strains of *Oxalobacter formigenes*.

strain OxGP, which fits with Group I based on rRNA sequences but is placed in Group II based on fatty acid profiles. Representative strains from both Group I and Group II contain both 12 and 14-carbon hydroxy fatty acids (Allison et al., 1985a). Comparisons of profiles of cellular proteins separated by polyacrylamide gel electrophoresis also support the distinctions between strains and groupings found by other methods (Jensen and Allison, 1994).

Results of both RFLP and PCR tests with oligonucleotide probes and primers also support the concept that *O. formigenes* strains can be separated into two main groups that fit with the above separations. Further separation of Group II strains into three subgroups has also been suggested (Sidhu et al., 1997a).

The mol% G + C of the DNA is: 48–51 (T_m).

Type strain: OxB, ATCC 35274.

GenBank accession number (16S rRNA): U49757.

Additional Remarks: GenBank accession numbers (16S rRNA) for other strains are U49754 (strain BA4), U49750 (strain HOxBLS), U49751 (strain SOx4), U49752 (strain OxWR), U49753 (strain OxGP), U49754 (strain OxCR), U49755 (strain OxC), U49756 (strain OxK), and U49758 (strain HC1).

2. **Oxalobacter vibrioformis** Dehning and Schink 1990, 320[VP] (Effective publication: Dehning and Schink 1989b, 82.)

vi.bri.o.for′mis. L. v. *vibrare* to vibrate; M.L. masc. n. *vibrio* that which vibrates; L. fem. n. *forma* shape, e.g., a curved cell; M.L. masc. adj. *vibrioformis* shaped like a curved cell.

The characteristics are as described for the genus, with the following additional features. Vibrioid rods 0.4 × 1.8–2.4 μm; motile by 1–2 polar flagella. Cells occur singly, in pairs, or in spiral chains. Growth occurs in freshwater or brackish water mineral medium. Temperature range: 18–35°C; optimum, 30–32°C. pH range, 5.6–8.3; optimum, 6.8–7.0. Catalase negative. No reduction of nitrate, sulfate, sulfite, thiosulfate, or sulfur. Indole is not formed from tryptophan. Does not hydrolyze urea, gelatin, or esculin. Acetate (1–2 mM) is required for growth. Formate is produced from oxalate. Cell yields are 1.2–1.3 g cell (dry wt) per mol of oxalate metabolized in growth medium that does not contain yeast extract and 1.6–1.7 g/mol in the presence of 0.05% yeast extract. The type strain was isolated from freshwater sediments, but similar strains have been isolated from marine sediments (Dehning and Schink, 1989b).

The mol% G + C of the DNA is: 51.6 ± 0.6 (T_m).

Type strain: WoOx3, DSM 5502.

Genus II. **Duganella** Hiraishi, Shin and Sugiyama 1997b, 1251[VP]

THE EDITORIAL BOARD

Du.ga.nel′ la. M.L. dim. ending *-ella;* M.L. fem. n. *Duganella* named after P.R. Dugan, the American microbiologist who isolated the organism.

Straight or slightly curved rods, 0.6–0.8 × 1.8–3.0 μm. **Motile by single polar flagellum.** Gram negative. Nonsporeforming. **Aerobic,** having a strictly respiratory metabolism with O_2 as the terminal electron acceptor. Nitrate is not used as a terminal electron acceptor. No chemolithotrophic growth occurs with H_2. **In liquid media, either of two types of growth may occur: amorphous flocs (occasionally fingerlike), or dispersed growth with little or no formation of flocs. Colonies on nutrient agar are pale yellow to straw-colored.** Mesophilic. Neutrophilic. Chemoorganotrophic. Growth occurs on organic media or on mineral media supplemented with simple organic compounds. **Catalase and oxidase positive. Acid is produced oxidatively from glucose** and other carbohydrates. **Starch, gelatin, casein, and urea are hydrolyzed.** Major cellular fatty acids are $C_{16:0}$, $C_{16:1}$; $C_{10:0\ 3OH}$ is the major hydroxy fatty acid. Ubiquinone-8 is the sole respiratory quinone. Putrescine and hydroxyputrescine are intracellular polyamines. The genus is classified in the class *Betaproteobacteria.* Isolated from sewage and polluted water.

The mol% G + C of the DNA is: 63–64.

Type species: **Duganella zoogloeoides** Hiraishi, Shin and Sugiyama 1997b, 1251.

DIFFERENTIATION OF THE GENUS *DUGANELLA* FROM OTHER GENERA

Characteristics differentiating the genus *Duganella* from other related genera are listed in Table BXII.β.18.

TAXONOMIC COMMENTS

The type strain of *D. zoogloeoides* (Dugan strain 115) was originally classified in the genus *Zoogloea* as a strain of *Zoogloea ramigera* (Friedman and Dugan 1968). This placement was questioned by Unz (1984), who found extensive phenotypic dissimilarities between strain 115 and the type strain of *Zoogloea ramigera.* Phylogenetic studies by Hiraishi et al. (1997b) based on 16S rDNA sequencing have shown that strain 115 is not closely related to the type strain of *Z. ramigera*, but is instead located in a different cluster, with its closest relatives being the genus *Telluria* and the species *Pseudomonas lemoignei* (for which a new genus, *Paucimonas*, has been proposed by Jendrossek (2001). In this 2nd edition of the *Manual*, the genus *Duganella* is classified in the class *Betaproteobacteria*, order *Burkholderiales*, and the family *Oxalobacteraceae.* Other genera belonging to this family include *Oxalobacter*, *Herbaspirillum*, *Janthinobacterium*, *Massilia*, and *Telluria*.

List of species of the genus Duganella

1. **Duganella zoogloeoides** Hiraishi, Shin and Sugiyama 1997b, 1251[VP]

   zo.o.gloe.o′ i.des. M.L. n. *Zoogloea* bacterial genus name; Gr. suff. *-oides* similar to; M.L. adj. *zoogloeoides* similar to *Zoogloea.*

   The characteristics are as described for the genus, together with the following additional information. Growth occurs on nutrient media or mineral media supplemented with simple organic compounds. Colonies on nutrient agar are glistening, convex with entire margins, viscous, and pale yellow to straw colored. Colonies may also appear tough, leathery, dry, and wrinkled. Denitrification does not occur. No growth factors are required, but yeast extract stimulates

TABLE BXII.β.18. Characteristics that differentiate members of the genus *Duganella* from the genera *Paucimonas, Herbaspirillum, Oxalobacter, Janthinobacterium, Telluria, Zoogloea,* and *Massilia*[a]

Characteristic	*Duganella*	*Herbaspirillum*[b]	*Janthinobacterium*[c]	*Massilia*[d]	*Oxalobacter*[e]	*Paucimonas*[f]	*Telluria*[g]	*Zoogloea*[h]
Anaerobic	−	−	−	−	+	−	−	−
Vibrioid to helical cells	−	+	−	−	−	−	−	−
Cell diameter >1.0 μm	−	−	+	−	−	−	−	+
Flagellation in liquid media:								
Single polar flagellum	+	−		+	−	+	+	+
1–3 flagella at one or both poles	−	+		−	−	−	−	−
Flocculent growth	v	−	−	+	−	−	−	+
Oxidase	+	+	+	−			W	+
Purple pigment (violacein) produced	−	−	d	−	−	−	−	−
Growth occurs on nutrient agar	+		+		−	−	−	W
Plant root-associated N_2 fixer	−	+	−	−		−	−	−
Arginine dihydrolase	−			+			−	−
Hydrolysis of starch	+	−	−	+		−	+	−
Hydrolysis of gelatin	+	−	+	+		−	+	+
Urease	+			−			+	+
Mol% G + C of the DNA	63–64	60–65	61–67	64		57–61	67–72	65

[a]Symbols: see standard definitions. W, weak; blank space, data either not reported or not applicable.

[b]Data from Baldani et al. (1986a), Baldani et al. (1996), and Lincoln et al. (1999).

[c]Data from Lincoln et al. (1999).

[d]Data from La Scola et al. (1998a).

[e]Data from Allison et al. (1985a), Dehning and Schink (1989b), and Lincoln et al. (1999).

[f]Data from Delafield et al. (1965), Mergaert et al. (1996), and Jendrossek (2001).

[g]Data from Bowman et al. (1993b).

[h]Data from Unz (1984).

growth. Forms an ester-like, sweet odor when grown with organic acids. The following carbohydrates are used as carbon sources, and acid is formed from them oxidatively: L-arabinose, D-xylose, D-glucose, D-fructose, D-galactose, D-mannose, maltose, sucrose, cellobiose, lactose, and glycogen. No growth or acid production occurs on D-ribose, L-rhamnose, glycerol, D-mannitol, D-sorbitol, or inositol. The following noncarbohydrates are used: pyruvate, citrate, succinate, fumarate, malate, malonate, tartrate, ethanol, alanine, aspartate, asparagine, glutamate, and proline. No or little growth with formate, acetate, propionate, butyrate, caproate, caprylate, methanol, propanol, benzoate, *p*-hydroxybenzoate, glycine, histidine, arginine, lysine, ornithine, or tryptophan. Putrescine and hydroxyputrescine are intracellular polyamines.

The mol% G + C of the DNA is: 63–64 (method not reported).

Type strain: Dugan 115, ATCC 25935, IAM 12670.
GenBank accession number (16S rRNA): D14256.
Additional Remarks: The type strain shows two types of colonies, designated A and B. Colonies of the A type are glistening, convex, viscous, and cream to straw colored. The organisms show dispersed growth rather than floc formation when cultured in complex peptone-containing media with shaking, but they do produce flocs when cultured in a chemically defined medium supplemented with organic acids. In contrast, colonies of the B type are tough, leathery, dry, wrinkled, and pale yellow. Flocs are produced in both complex and chemically defined media and are amorphous, although occasionally flocs with fingerlike projections occur. The A and B types give 93–102% DNA–DNA hybridization to each other.

Genus III. **Herbaspirillum** Baldani, Baldani, Seldin and Döbereiner 1986a, 90[VP] emend. Baldani, Pot, Kirchhof, Falsen, Baldani, Olivares, Hoste, Kersters, Hartmann, Gillis and Döbereiner 1996, 808

JOSÉ IVO BALDANI, VERA LÚCIA DIVAN BALDANI AND JOHANNA DÖBEREINER

Her.ba.spi.ril' lum. L. fem. n. *herba* herbaceous, seed-bearing plant that does not produce persistent woody tissue; M.L. dim. neut. n. *spirillum* small spiral; *Herbaspirillum* small, spiral-shaped bacteria from herbaceous, seed-bearing plants.

Cells are generally **vibrioid**, sometimes spirilloid, approximately **0.6–0.7 μm in diameter**. The cell length varies from 1.5–5.0 μm depending on the culture medium. **Gram negative.** Motile, having 1–3 flagella at one or both poles. The organisms have a strictly **respiratory type of metabolism**, and sugars may be oxidized but not fermented. **The three named species presently included in the genus have the ability to fix atmospheric N_2 under microaerobic conditions** and grow well with N_2 as the sole nitrogen source, even in the presence of 10% sucrose. **Oxidase and urease positive.** Catalase variable or weak. Salts of organic acids such as malate, pyruvate, succinate and fumarate are the favored carbon source for NH_4^+ or N_2-dependent growth. Other carbon sources, such as glycerol, mannitol, D-glucose, and sorbitol, but not sucrose, are also catabolized. Optimal temperature, 30–34°C. **Optimal pH, 5.3–8.0.** Colonies on JNFb agar plates containing three times the usual concentration of bromothymol blue are smooth and white with blue or green centers after one week. Occur mainly in association with **graminaceous plants endophytically colonizing roots, stems and leaves**.

The mol% G + C of the DNA is: 60–65.
Type species: **Herbaspirillum seropedicae** Baldani, Baldani, Seldin and Döbereiner 1986a, 90 emend. Baldani, Pot, Kirchoof, Falsen, Baldani, Olivares, Hoste, Kersters, Hartmann, Gillis and Döbereiner 1996, 808.

FURTHER DESCRIPTIVE INFORMATION

Morphological features The flagellar arrangement is shown in Figs. BXII.β.13 and BXII.β.14. No lateral flagella have been observed for the species. Cells become very motile when close to air bubbles. Cells become elongated when grown in a semisolid medium containing glucose, galactose, arabinose, mannitol, or glycerol as the sole carbon source in the presence of 20 mM NH_4Cl (Figs. BXII.β.15 and BXII.β.16) (Baldani et al., 1992). With monosaccharides, *H. rubrisubalbicans* cells are more elongated and thinner, whereas *H. seropedicae* cells are wider. With organic acids such as malate, succinate, α-ketoglutarate, and fumarate, these characteristics have not been observed. The size of *H. seropedicae* and *H. rubrisubalbicans* cells are also different when they endophytically colonize sugarcane plant tissues. *H. seropedicae* has a cell size of 1.7 × 0.52 μm, whereas *H. rubrisubalbicans* cells are 2.1 × 0.45 μm (Olivares, 1997).

Cultural characteristics In semisolid JNFb medium[1], *H. seropedicae* and *H. rubrisubalbicans* form a fine white pellicle below the surface of the medium after incubation for 48 h at 30–34°C. The cells remain motile and vibrioid even after 1 week. Very pronounced swarming has been observed for *H. seropedicae* grown on nutrient agar (0.8% agar) plates at 35°C. *H. seropedicae* and *H. rubrisubalbicans* can grow and fix nitrogen in semisolid NFb medium, the medium routinely used to isolate *Azospirillum lipoferum* and *A. brasilense*. On this medium, the pellicles are similar to those of *Azospirillum*, except that the larger cell size of the latter differentiates *Herbaspirillum* cells under the microscope. No nitrogen fixation has been observed for either diazotrophic species grown at temperatures above 38°C, although growth can be observed at 41°C with inorganic nitrogen sources (Baldani et al., 1992).

The colonies are small, smooth, and whitish with blue or green centers when grown on JNFb agar plates containing three times

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1. JNFb medium consists of L-malic acid, 5.0; K_2HPO_4, 0.6; KH_2PO_4, 1.8; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; $CaCl_2 \cdot 2H_2O$, 0.02; trace element solution ($Na_2MoO_4 \cdot 2H_2O$, 0.2 g; $MnSO_4 \cdot H_2O$, 0.235 g; H_3BO_3, 0.28 g; $CuSO_4 \cdot 5H_2O$, 0.008 g; $ZnSO_4 \cdot 7H_2O$, 0.024 g; distilled water, 1000 ml), 2.0 ml; bromthymol blue (0.5% aqueous solution (dissolve in 0.2 N KOH)), 2.0 ml; FeEDTA (1.64% solution), 4.0 ml; vitamin solution (biotin, 0.01 g; pyridoxine, 0.02 g; distilled water, 1000 ml), 1.0 ml; KOH, 4.5; pH adjusted to 5.8 with KOH. Bring the final volume to 1000 ml with distilled water. For a semisolid medium, add 1.75 to 1.9 g agar/l (agar should be dissolved before distribution into vials); for a solid medium, add 15.0 g agar/l.
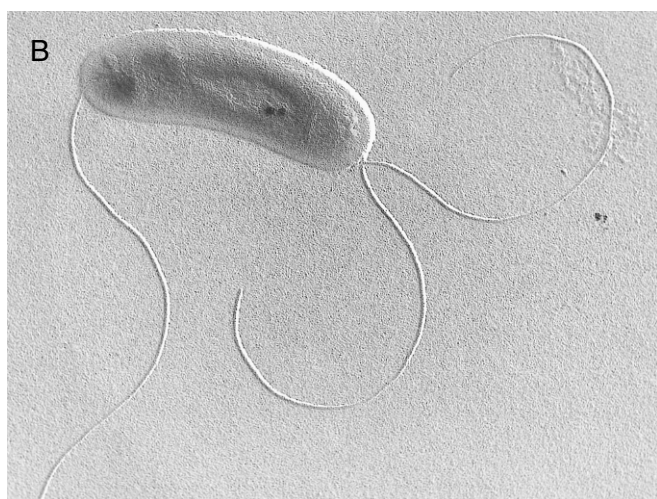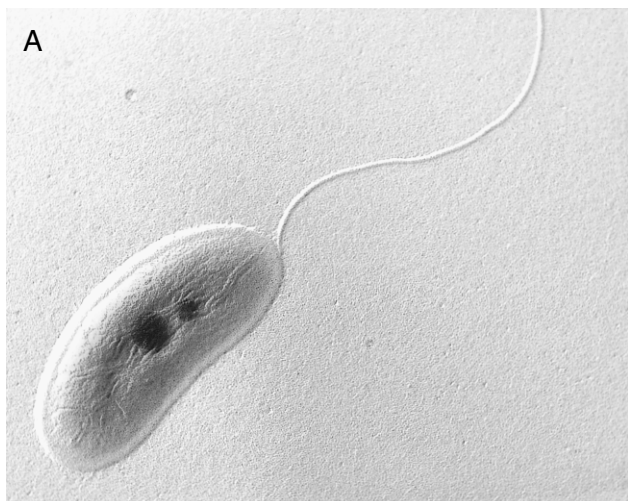
FIGURE BXII.β.13. *Herbaspirillum seropedicae* ATCC 35892 cells grown in nutrient broth and showing single (*A*) and double (*B*) polar flagella. (× 11,000) (Courtesy of N.R. Krieg).

FIGURE BXII.β.14. *Herbaspirillum rubrisubalbicans* ATCC 19308 cells grown in nutrient broth and showing several polar flagella (× 12,000) (Courtesy of F.O. Olivares).

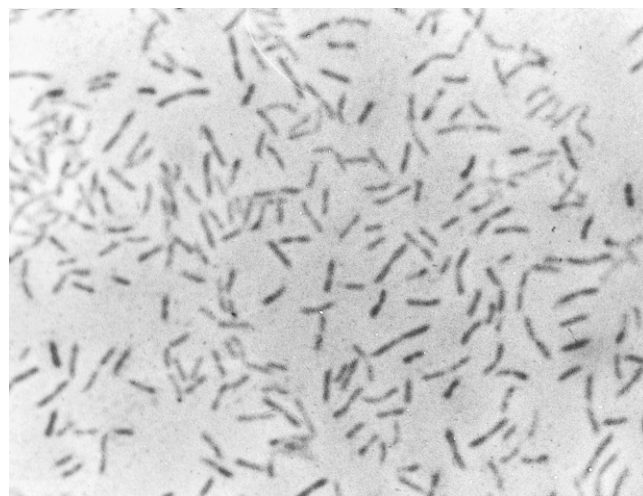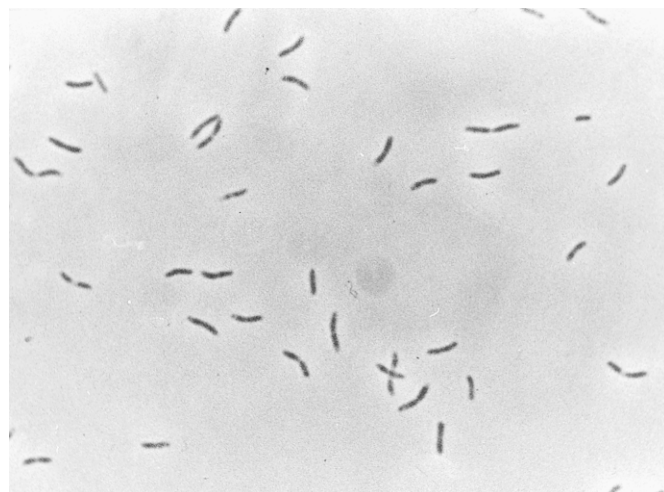FIGURE BXII.β.15. *Herbaspirillum seropedicae* ATCC 35892 cultured in semisolid medium containing glucose as the sole carbon source and 20 mM NH_4Cl.

FIGURE BXII.β.16. *Herbaspirillum rubrisubalbicans* ATCC 19308 cultured in semisolid medium containing glucose as the carbon source and 20 mM NH_4Cl.

the usual amount of the bromothymol blue indicator. On BMS agar plates, colonies are moist and small with brown centers. (The recipe for BMS agar is given in the chapter on the genus *Azospirillum* located in the family *Rhodospirillaceae*.)

There is no growth or nitrogen fixation activity in liquid nitrogen-free medium under air (20.9% O_2). However, nitrogenase activity can be detected under an air when grown in liquid JNFb or NFbHP medium supplemented with L-glutamate and L-glutamine, but not with L-serine, L-alanine, or ammonium chloride. (The recipe for NFbHP medium is given in the chapter on the genus *Azospirillum* in the family *Rhodospirillaceae*.) Nitrogen fixation occurs only when the nitrogen source is exhausted from the culture medium. Other nitrogen sources, such as L-histidine, L-lysine, L-arginine, and the amines methylammonium chloride, tetramethylammonium chloride, and ethylenediamine chloride, do not support growth or nitrogen fixation by *H. seropedicae* (Klassen et al., 1997). Strains from this species also assimilate or dissimilate nitrate to nitrite under oxygen limitation, but no NO_3^--

dependent anaerobic growth or visible gas production from nitrate is observed either in solid or semisolid medium. Small amounts of N_2O are detected when 10% acetylene is added. Most strains from *H. rubrisubalbicans* reduce nitrate to nitrite, but denitrification has not been observed.

Compounds that can serve as sole carbon and energy sources for N_2-dependent growth of *H. seropedicae* and *H. rubrisubalbicans* strains include malate, succinate, citrate, α-ketoglutarate, fumarate, pyruvate, *trans*-aconitate, mannitol, glycerol, sorbitol, glucose, galactose, and L-arabinose. *N*-acetylglucosamine is also used as a sole carbon source for N_2-dependent growth by *H. seropedicae* strains. On the other hand, *meso*-erythritol is used by strains of *H. rubrisubalbicans* when ammonium chloride is added to the medium.

Biochemical tests (API 50CH, 50 A, and 50AA kits) reveal that catabolizabled carbon sources can be used to differentiate these species. All tested strains use the following substrates: glycerol, D- and L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, mannitol, sorbitol, xylitol, D-lyxose, DL-lactate, DL-glycerate, DL-3-hydroxybutyrate, pyruvate, citrate, succinate, fumarate, L-leucine, L-threonine, L-aspartate, L-glutamate, α-ketoglutarate, adonitol, D-galactose, L-fucose, D-and L-arabitol, gluconate, 2-ketogluconate, propionate, isobutyrate, *n*-valerate, isovalerate, D- and L-malate, *meso*-tartrate, butyrate, DL-4-aminobutyrate, aconitate, mesoaconitate, *p*-hydroxybenzoate, L-proline, D-arabinose, 5-ketogluconate, acetate, caprate, amylamine, D-fucose, and tryptamine (Baldani et al., 1996). Whether all of these carbon sources can support N_2-dependent growth has yet to be determined. On the other hand, all tested strains are unable to use the following carbon sources: arbutin, salicin, D-cellobiose, maltose, sucrose, trehalose, inulin, D-tartrate, D-raffinose, starch, L-phenylalanine, DL-5-aminovalerate, betaine, glutarate, L-histidine, L-sorbose, dulcitol, esculin, glycogen, phthalate, oxalate, and maleate.

Strains from this genus have a strictly respiratory metabolism, and sugars are oxidized but not fermented. Acid is produced from L-arabinose under N_2-fixation conditions, although it has also been observed from other carbon sources, such as glucose, fructose, galactose, mannitol, lactose, glycerol, and sorbitol, when using biochemical tests. The efficiency of N_2-fixation for *H. seropedicae* strains as evaluated in semisolid NFb medium is 12–15 mg N_2 per g DL-malate or 13 mg N_2 per g mannitol. Vitamins or other growth substances are not required. The plant-growth substance indoleacetic acid has been detected in strains of *H. seropedicae* and *H. rubrisubalbicans* when tryptophan is added.

Genetic features Many of the *nif* genes (*nif A, B, HDK*), as well as *glnA* and *B*, and *ntrBC* genes have already been identified in the chromosome of *H. seropedicae* species (Pedrosa et al., 1997). Plasmids have also been detected for some *H. seropedicae* strains, but no function has been ascribed to them (Pedrosa, personal communication).

Plasmids of the IncP1 incompatibility group are stable in *Herbaspirillum seropedicae* and can be transferred to this species by conjugation, electroporation, and transformation (Vande Broek et al., 1996; Pedrosa et al., 1997). Plasmids from this group have also been transferred by conjugation to a strain of *H. rubrisubalbicans* (Gitahy et al., 1997).

Antigenic features Polyclonal antibodies against whole cells of the type species of *H. seropedicae* (strain Z67) and against *H. rubrisubalbicans* strain M4 have been very useful for discriminating the plant tissue colonization by *H. seropedicae* and *H. rubrisubalbicans* when applying immunogold labeling (Olivares et al., 1997). Purified antisera can discriminate among strains of both species by ELISA, and the immunotrapping technique using antibody-specific for *H. seropedicae* has permitted isolation of strains from sugarcane roots grown in the field (Reis et al., 1998).

Antibiotic susceptibility and resistance Strains of *H. seropedicae* and *H. rubrisubalbicans* are resistant to nalidixic acid, penicillin, and low concentrations of novobiocin and rifampicin. They are sensitive to several antibiotics, including streptomycin, kanamycin, spectinomycin, tetracycline, and chloramphenicol (Ureta et al., 1995; Baldani et al., 1986a).

Ecology The majority of the isolates of the genus *Herbaspirillum* has been isolated from graminaceous plants and show the ability to fix nitrogen. On the other hand, it has been shown that strains of *H. rubrisubalbicans* are mild pathogenic agents to some susceptible sugarcane varieties, causing "mottled stripe disease," mainly in crops highly fertilized with nitrogen. Experiments carried out in Brazil have shown that all commercial varieties are resistant to this disease, and neither *H. seropedicae* nor *H. rubrisubalbicans* strains cause the characteristic symptoms when artificially inoculated into leaves by injection (Olivares et al., 1997). In addition, strains of *H. seropedicae* and *H. rubrisubalbicans* cause "red stripe disease" in *Pennisetum purpureum* as well as in sorghum bicolor, although symptoms are very mild in sorghum leaves inoculated artificially with these bacteria (Pimentel et al., 1991; Olivares et al., 1997). However, symptoms are not observed in maize plants inoculated artificially with *H. seropedicae* and *H. rubrisubalbicans* strains (Olivares, 1997).

The ecological distribution of *Herbaspirillum seropedicae* and *H. rubrisubalbicans* has so far been restricted to tropical areas, where they have been found in numbers varying from $10^2–10^7$ cells/g of fresh plant tissues. Strains of *H. seropedicae* were first isolated from washed, surface-sterilized roots of maize, sorghum, and rice grown in two different soils in Rio de Janeiro State, as well as from maize plants grown in a Cerrado soil in Brasilia, DF, Brazil (Baldani et al., 1986a). A few isolates have been obtained from rhizosphere soil (Baldani et al., 1986a). Since it is now known that *H. seropedicae* does not survive well in soil and could not be found in 10 different soil samples free of roots collected around Seropédica (Olivares et al., 1996), we suspect that small root pieces were present in the rhizosphere soil utilized by Baldani et al. (1986a). In addition, *H. seropedicae* has been isolated from roots and stems of *Saccharum* spp. and roots of *Echinola crusgalli*, *Pennisetum purpureum*, *Panicum maximum*, *Digitaria decumbens*, *Brachiaria decumbens*, and *Melinis minutiflora* (Olivares et al., 1996). In contrast, *H. rubrisubalbicans* has been isolated from roots, stems, and leaves of sugarcane plants as well as from roots of *Digitaria insularis*, a weed plant grown in the sugarcane field. No isolates of *H. seropedicae* and *H. rubrisubalbicans* could be found in many samples from seven different plant families: Compositae, Molluginaceae, Sterculaceae, Cyperaceae, Portulacaceae, Leguminosae, and Cucurbitaceae (Olivares et al., 1996). One isolate resembling *H. rubrisubalbicans* has been identified in banana plant material (Weber et al., 1997). One isolate of *H. seropedicae* and one resembling *H. rubrisubalbicans* have been identified in banana plant material (Weber et al., 1999).

Electron-microscopic analysis shows that *H. rubrisubalbicans* colonizes the xylem, intercellular, and substomatal cavities of a mottled-stripe-susceptible sugarcane variety, in which the bacteria are restricted to microcolonies encapsulated within membranes of plant cell origin (Olivares et al., 1997; Fig. BXII.β.17). Colonizations in intercellular spaces and xylem have also been found
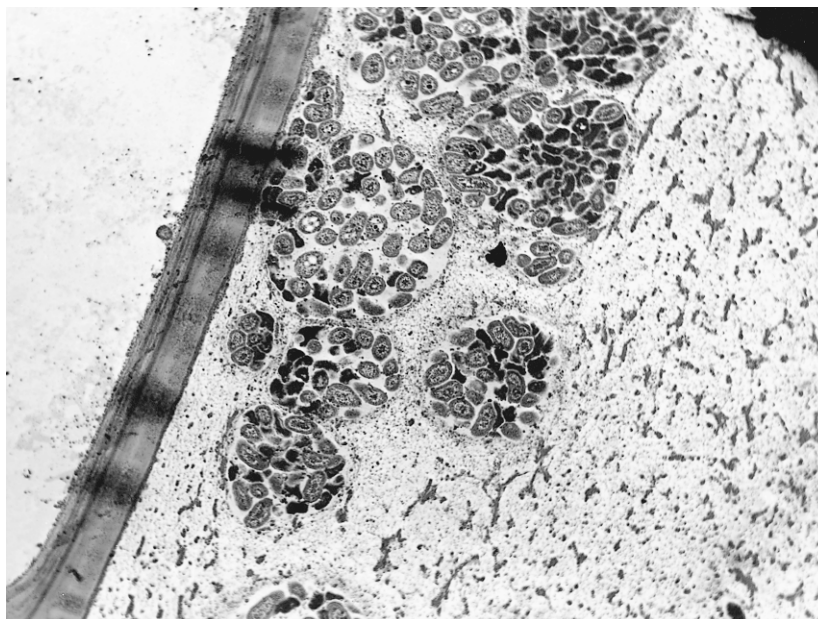
FIGURE BXII.β.17. Transmission electron micrograph (TEM) of xylem vessel from the leaf of a sorghum plant inoculated with *Herbaspirillum rubrisubalbicans* ATCC 19308 showing the microcolonies close to the walls of the vessels and that are surrounded by host-derived gums. (Reprinted with permission from E.K. James and F.L. Olivares, Critical Reviews in Plant Sciences *17:* 77–119, 1997, ©CRC Press, Boca Raton.)

for *Herbaspirillum* spp. in sugar cane roots (Olivares et al., 1997). *H. seropedicae* has also been localized within intercellular cavities of rice seeds and roots (Baldani et al., 1994).

Field inoculation experiments have shown yield increases of maize inoculated with *H. seropedicae* strains (Pereira et al., 1988). Increase of dry weight and grain yield has been observed in rice plants inoculated with selected strains of *H. seropedicae* (Döbereiner and Baldani, 1998).

ENRICHMENT AND ISOLATION PROCEDURES

Serial dilutions (0.1 ml) of root, stem, or leaf samples are inoculated into 10-ml cotton-plugged serum vials containing 5 ml of semisolid JNFb medium and incubated for 1 week at 32°C. Smashed pieces (5–8 mm long) of plant tissues can also be used, but the incubation time should be only 40–48 h. For vials exhibiting a fine white pellicle, cells are examined under the microscope for the presence of small, curved rods (0.6–0.7 × 4–6 μm) that move faster close to air bubbles. After a second transfer to JNFb semisolid medium and incubation for 24–48 h, cultures are streaked out on a solid JNFb medium containing 20 mg/l yeast extract and three times the bromothymol blue concentration of the JNFb medium. On these plates, *H. seropedicae* and *H. rubrisubalbicans* form small, moist colonies with a green or dark blue center (different from the white colonies of *Azospirillum lipoferum* and *A. brasilense*). For final purification, single colonies are again transferred to the JNFb semisolid medium, and cells from the typical pellicle are streaked onto BMS agar plates, on which moist, smooth and small brownish colonies develop. Colonies are selected and stored for further identification (Döbereiner et al., 1995).

MAINTENANCE PROCEDURES

Stock cultures can be maintained on JNFb or BMS medium under a layer of sterilized mineral oil in tubes tightly sealed with rubber caps. Under these conditions, *H. seropedicae* remains viable at room temperature for more than 12 years.

For routine use in the laboratory, it is recommended to maintain duplicate vials and subculture strains from time to time (e.g., every 6 months, with maintenance at room temperature). Strains can also be maintained in glycerol at −20°C by mixing equal volumes of sterilized glycerol and washed, resuspended cells from a 48-h-old culture grown in liquid JNFb medium (containing 20 mg of yeast extract and 5 mM ammonium chloride or sodium glutamate).

Strains can also be kept lyophilized for many years. Cells grown on slant JNFb medium, with malic acid replaced by D-glucose, for 48–72 h at 30°C are suspended in 2 ml of a 10% sucrose solution and 5% peptone in 100 ml water. Aliquots (0.2 ml) are then distributed into lyophilization ampoules and lyophilized.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Meso-erythritol and N-acetylglucosamine as carbon sources for growth that is dependent on nitrogen fixation or inorganic nitrogen A loopful of culture grown in semisolid JNFb medium is inoculated into a vial of semisolid JNFb medium (in which malate is replaced by 0.5% *meso*-erythritol plus 1 g of NH_4Cl or 0.5% *N*-acetylglucosamine). *H. seropedicae* forms a fine pellicle on the surface of a semisolid medium containing *N*-acetylglucosamine after incubation at 32°C for 48 h, but no growth is observed in the presence of *meso*-erythritol. On the other hand, *H. rubrisubalbicans* forms a typical pellicle in the presence of *meso*-erythritol plus NH_4Cl, but not with *N*-acetylglucosamine as a carbon source. API galleries (API 50CH, API 50AO, and API 50AA; bioMérieux, Montalieu-Vercieu, France) can also be used to distinguish *H. seropedicae* from *H. rubrisubalbicans*.

Oligonucleotide probes Probe HS (5′-GTC CCG GTT TTT GCA TCG A-3′) and probe HR (5′-TAG TCG GTT TTT GCA

TCG A-3′) are species-specific for a highly variable stretch of helix 55–59 of the 23S rRNA of *H. seropedicae* and *H. rubrisubalbicans*. Bulk nucleic acids are isolated from strains, cultivated overnight in DYGS liquid medium, and transferred to a positively charged nylon membrane via spot blotting. Hybridization with radioactive or non-radioactive DIG-labeled probes is performed for 2–12 h (radioactive) or 16 h (nonradioactive) at 52°C according to the method of Kirchhof et al. (1997b). Hybridization signals are detected by autoradiography.

DIFFERENTIATION OF THE GENUS *HERBASPIRILLUM* FROM OTHER GENERA

The morphological, physiological and genetic characteristics that differentiate *Herbaspirillum* from other diazotrophic species within the class *Betaproteobacteria* and from *Azospirillum brasilense* are shown in Table BXII.β.19.

TAXONOMIC COMMENTS

This genus *Herbaspirillum* constitutes a separate rRNA cluster within the class *Betaproteobacteria*. Comparison of many bacteria within this group based on the $T_{m(e)}$ values of the DNA–rRNA hybridization experiments has shown that *Herbaspirillum* has a very close relationship to the genus *Janthinobacterium*. However, *Janthinobacterium* has the lowest $T_{m(e)}$ value within this rRNA cluster and because of its phenotypic distinction from the other members of this rRNA branch, including its inability to fix nitrogen, it has been maintained as a separate genus. The DNA–DNA hybridization experiments also confirm the results, since they show a very low degree of similarity with the *Herbaspirillum* species. More recently, a comparison of the 16S rRNA full sequence from *H. seropedicae* with those of several other non-nitro-gen-fixing bacteria from the *Betaproteobacteria* has shown that this species, along with *Oxalobacter formigenes*, forms a separate lineage in the *Betaproteobacteria* (Sievers et al., 1998).

Other nitrogen-fixing bacteria are also found within the *Betaproteobacteria*, including the species *Burkholderia vietnamiensis*, *Azoarcus indigens*, and *Alcaligenes faecalis*. Although they share the ability to fix nitrogen, they have several other distinguishable phenotypes and genetic aspects that maintain them as separate species from the genus *Herbaspirillum*. *B. vietnamiensis* is able to use glucose, caprate, and itaconate as carbon sources, as does *Herbaspirillum*. However, in contrast to the latter genus, it is urease negative, the cells are not curved, the optimal temperature for growth is much lower, and it has been isolated mainly from rhizosphere rice roots. The mol% G + C is 66.9–68.1, which is higher than the 60–65 mol% G + C value established for *Herbaspirillum* species. The genus *Herbaspirillum* has some characteristics similar to those of *Azoarcus indigens*, such as cell shape, vibrioid movement, and an ability to use malic acid. However, *A. indigens* has a much higher oxygen tolerance for N_2 fixation (6.5%), in contrast to the 2.5% observed for the *Herbaspirillum*. In addition, it has a much higher optimal temperature for growth (40°C) and has been found associated only with Kallar grass plants. Although the $T_{m(e)}$ values show that they belong to the same rRNA superfamily, they are quite separate based on the DNA–rRNA hybridization dendrogram and therefore should not be included in the same genus. The other nitrogen-fixing species within the *Betaproteobacteria* has been identified as *Alcaligenes faecalis*. It has also been isolated from rice plants (You et al., 1991) and has an oxygen tolerance for N_2 fixation, size, and mol% G + C very close to those of *Herbaspirillum*.

Due to the similar shape, vibrioid movement, and mol% G

TABLE BXII.β.19. Differential morphological and physiological characteristics of the genus *Herbaspirillum*, selected diazotrophic species within the class *Betaproteobacteria*, and *Azospirillum brasilense* (class *Alphaproteobacteria*)[a]

Characteristic	*Herbaspirillum*	*Azoarcus indigens*[b]	*Azospirillum brasilense*[c]	*Burkholderia vietnamiensis*[d]
Arrangement of flagella:				
Location	Polar	Polar	Polar[e]	Polar or bipolar
Number	1–3	1	1[e]	1 or tuft
Vibrioid cell shape	+	+	+	−
Cell width, μm	0.5–0.7	0.5–0.7	1.0–1.2	0.3–0.8
Urease	+	+	+	−
Denitrification	−	−	v	+
Optimal growth temperature, °C	30–34	40	37	28
Carbon sources:				
D-Glucose	+	+	+	−
Sucrose	+	−	−	−
meso-Tartrate	+	d	nd	d
Caprate	+	−	nd	+
Itaconate	+	+	nd	+
Maximum O_2 tension (%) for N_2-fixation [f]	2.5	6.5	2.0	3.0
Habitats:				
Plant tissues	+	+	+	−
Soil	−	−	+	+
Clinical materials	−	−	−	−[g]
Mol% G + C of the DNA	60–65	66.6	70–71	66.9–68.1

[a]For symbols, see standard definitions, nd, not determined.

[b]Data from Reinhold-Hurek et al. (1993b).

[c]Date from Döbereiner (1991).

[d]Data from Gillis et al. (1995).

[e]In liquid medium, the cells possess only single polar flagella, but on agar media, several lateral flagella of shorter wavelength also occur.

[f]According to the experiments carried out by Vande Broek et al. (1996).

[g]A few strains are of clinical origin.

+ C content of DNA, *Herbaspirillum seropedicae* was provisionally placed into the genus *Azospirillum* (Baldani et al., 1984). However, results from RNA–RNA hybridization experiments comparing several strains of *H. seropedicae* with the three known species of *Azospirillum* and other possibly related N$_2$-fixing bacteria yielded very low RNA similarity values (<20%) (Falk et al., 1986). These differences were also confirmed by analysis of the SDS-PAGE membrane-protein pattern of strains of both genera: a characteristic major band at 37.5 kDa occurs only in *H. seropedicae* strains (Dianese et al., 1989). rRNA sequence analysis has shown that the genus *Herbaspirillum* belongs to the *Betaproteobacteria*, whereas the *Azospirillum* species were grouped into the *Alphaproteobacteria*. Even though *H. seropedicae* species show some physiological and morphological characteristics similar to those of *Azospirillum brasilense*, the genetic data show that it should not be included in the genus *Azospirillum*.

The genus *Herbaspirillum* was expanded when biochemical, DNA–rDNA, and DNA–DNA hybridization studies showed that *Pseudomonas rubrisubalbicans*, the causative agent of the "mottled strip" disease in sugarcane, has a high degree of rRNA similarity to *Herbaspirillum* and should therefore be included in this genus (Gillis et al., 1991). These data were confirmed by additional features of *P. rubrisubalbicans*, including its ability to fix nitrogen, culminating with the reclassification of the species as *Herbaspirillum rubrisubalbicans* (Baldani et al., 1996).

DNA–DNA hybridization studies have shown that there are three distinct groups, i.e., species, of bacteria within the *Herbaspirillum* genus. High values of DNA–DNA hybridization (71–100%) have been observed for *H. seropedicae* and *H. rubrisubal-*

bicans, and the full sequences of the 16S rRNAs of the type strains from both species show a 99.2% similarity (Kirchhof et al., 2001).

On the basis of physiological properties, phylogenetic analysis comparing 16S rDNA sequences and DNA–DNA hybridization experiments with chromosomal DNA, Kirchhof et al. (2001) created a new species, *Herbaspirillum frisingense*, for nitrogen-fixing bacteria isolated from the C4-fibre plants, *Spartina pectinata, Miscanthus sinensis, Miscanthus sacchariflorus,* and *Pennisetum purpureum.* Nitrogen-fixing capability was examined by PCR amplification of the *nifD* gene and an acetylene reduction assay and was found with all isolates tested. The 16S rDNA sequence similarity to the other two *Herbaspirillum* species is 98.5–99.1%.

FURTHER READING

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DIFFERENTIATION OF THE SPECIES OF THE GENUS *HERBASPIRILLUM*

The differential characteristics of the species *Herbaspirillum* are indicated in Table BXII.β.20. Other characteristics of the species are presented in Table BXII.β.21.

Although the 16S rDNA sequence analysis of *H. seropedicae* and *H. rubrisubalbicans* shows a high degree of similarity between

the two (99.2% Kirchhof et al., 2001), analysis of the hypervariable region of the 23S rDNA shows a higher degree of variation, allowing for the design of probes to differentiate between *H. seropedicae* and *H. rubrisubalbicans* (Kirchhof et al., 1997b).

List of species of the genus Herbaspirillum

1. **Herbaspirillum seropedicae** Baldani, Baldani, Seldin and Döbereiner 1986a, 90[VP] emend. Baldani, Pot, Kirchoof, Falsen, Baldani, Olivares, Hoste, Kersters, Hartmann, Gillis and Döbereiner 1996, 808.

   se.ro.ped′ i.cae. L. gen. n. *seropedicae* of Seropédica, Rio de Janeiro, Brazil, where the species was first isolated.

   The characteristics are as described for the genus and in Tables BXII.β.20 and BXII.β.21.

   Cells are vibrioid and sometimes spirilloid and become motile when close to O$_2$ sources. Two polar flagella are generally found at one or both poles, and pronounced swarming is observed on soft nutrient agar at 35°C. Oxidase, urease, and catalase positive. All strains except LMG 2284 fix nitrogen under microaerobic conditions and grow well with N$_2$ as a sole nitrogen source. Optimal pH and temperature for N$_2$-dependent growth in semisolid JNFb medium are 5.8 and 34°C, respectively. N$_2$-fixation can also occur in liquid medium when L-glutamate or L-glutamine

used as the sole nitrogen source is completely exhausted. Colonies on JNFb agar plates containing three times the usual concentration of bromothymol blue are small and moist with a green or dark blue center.

Salts of organic acids such as malate, fumarate, pyruvate, succinate, and *trans*-aconitate are favored carbon sources for both N$_2$- and NH$_4$-dependent growth, as are glucose, galactose, and L-arabinose. Fructose is not used for N$_2$-dependent growth. Growth and N$_2$ fixation occur in the presence of 10% sucrose. Vitamins and growth substances are not required. Sensitive to chloramphenicol, tetracycline, gentamicin, kanamycin, and streptomycin and resistant to penicillin, nalidixic acids, and low concentrations of novobiocin and rifampicin.

Habitats are roots, stems, and leaves of many members of the family Gramineae, but so far strains have not been isolated from soil, unless sorghum plants are used as trapping hosts. When artificially inoculated into leaves of sor-

TABLE BXII.β.20. Characteristics differentiating the species of the genus *Herbaspirillum*[a]

Characteristic	*H. seropedicae*	*H. frisingense*	*H. rubrisubalbicans*
Usual polar flagella arrangement	2 at one or both poles	Mostly two, occasionally 1 or 3; unipolar	Several polar
Carbon sources used for growth:			
meso-Erythritol	−	−	+
N-Acetylglucosamine, L-rhamnose, *meso*-inositol	+	+[b]	−
Adipate, pimelate, azelate, suberate, L-tartrate	−	−[c]	−
Hybridization with probes:			
HS	+	−	−
HR	−	−	+
Optimal temperature for growth, °C	34	30–37	30
Causes mottled stripe disease symptoms in susceptible sugar cane variety B-4362	−	−	+

[a]For symbols, see standard definitions.

[b]Positive only for *N*-acetylglucosamine.

[c]Only tested for adipate.

TABLE BXII.β.21. Additional characteristics of the species of the genus *Herbaspirillum*[a]

Characteristics	*H. seropedicae*	*H. frisingense*	*H. rubrisubalbicans*
Nitrogenase activity	+	+	+
Oxidase and urease	+	+	+
Cell length, μm	1.5–5.0	1.4–1.8	1.5–2.1
Hydrolysis of starch and gelatin	−	−	−
NO_3^- to NO_2^-	+	+	+
NO_2^- to N_2O	∓[b]	−	−
Optimal pH for growth	5.3–8.0	6.0–7.0	5.3–8.0
Maximum growth temperature (°C) in presence of organic nitrogen sources	38	37	41
$^{15}N_2$ incorporation into cells grown in semisolid media	+	nd	+
Growth in presence of 2% NaCl	−	nd	nd
Growth in presence of 10% sucrose	+	nd	+
Carbon sources used for growth (API tests):			
Sucrose, maltose, D-raffinose	−	−	−
Galactose, gluconate, adonitol, D-glucose,	+	+	+
D-Fructose,[c] mannitol, L-malate, pyruvate, citrate, succinate, 5-ketogluconate, acetate	+	+	+
Survival in soil	Poor	nd	Poor
Habitat	Tissues of several grasses	Tissue of C-4 fiber plants	Only leaves of sugar cane
Mol% G + C of DNA	64–65	61–65	62–63

[a]For symbols, see standard definitions; nd, not determined; ∓, several strains positive but the majority of strains negative.

[b]Several strains are able to reduce nitrite to N_2O, but the majority of strains are negative.

[c]All species grow poorly in D-fructose when dependent on nitrogen fixation.

ghum, *H. seropedicae* causes a mild symptom of red stripe disease.

The mol% G + C of the DNA is: 64–65 (T_m).

Type strain: Z67, ATCC35892, BR 11175, DSM 6445.

GenBank accession number (16S rRNA): Yl0146.

Additional Remarks: Reference strains: ATCC 35893 (BR11177, Z78); ATCC 35894 (BR 11178, Z152).

2. **Herbaspirillum frisingense** Kirchhof, Eckert, Stoffels, Baldani, Reis and Hartmann 2001, 166[VP]

fri.sin.gen' se. frisingense L. gen. n. of Frisinga, now known as Freising, Germany, town where the species was first isolated.

The characteristics are as described for the genus.

Cells are vibrioid rods, but smaller and have a single polar flagellum. This group forms a fine pellicle on the surface of the semisolid NFb or JNFb medium quite similar to that of the other species. Colonies on JNFb agar plates containing three times the concentration of bromthymol blue are small and moist with a green or dark blue center. Oxidase, urease, and catalase positive. Gelatin is not hydrolyzed. All strains catabolize *N*-acetylglucosamine, arabinose, caprate, citrate, glucose, gluconate, malate, mannose, mannitol, and phenylacetate, but not maltose or adipate, as determined by biochemical analyses. All isolates reduce nitrate to nitrite but not to N_2. Possess a cytochrome oxidase. N_2-dependent growth (subsurface pellicle) was also observed with arabinose, *N*-acetylglucosamine, malate, mannitol, and glucose. In contrast to *H. seropedicae*, these isolates do not use L-rhamnose or inositol as sole carbon sources. They also differ from *H. rubrisubalbicans* by the ability to

use *N*-acetylglucosamine but not *meso*-erythritol in the presence of NH₄Cl. Based on the sequence of a highly variable 23S rDNA stretch within domain III, an oligonucleotide probe named beta 20 (5′-GAT ACA AGA ACC GGG AC-3′) is used to distinguish *H. frisingense* from the other species of the genus.

Habitats are roots, stems, and leaves of the C-4 graminaceous plants *Spartina pectinata*, *Miscanthus sinensis*, and *Miscanthus sacchariflorus* grown in Germany and *Pennisetum purpureum* grown in Brazil.

The mol% G + C of the DNA is: 61–65 (T_m).

Type strain: DSM 13128, GSF30.

GenBank accession number (16S rRNA): AJ238358.

Additional remarks: Reference strains: DSM 13130 (strain Mb11) and ATCC 35894 (strain 75B).

3. **Herbaspirillum rubrisubalbicans** (Christopher and Edgerton 1930) Baldani, Pot, Kirchhof, Falsen, Baldani, Olivares, Hoste, Kersters, Hartmann, Gillis and Döbereiner 1996, 809^VP (*Pseudomonas rubrisubalbicans* (Christopher and Edgerton 1930) Krasil'nikov 1949, 379; *"Phytomonas rubrisubalbicans"* Christopher and Edgerton 1930, 266.)

ru.bri.sub.al′ bi.cans. L. adj. *ruber* red; adj. *subalbicans* whitish; M.L. adj. *rubrisubalbicans* red-whitish, referring to the symptoms of mottled stripe disease.

Cells are slightly curved rods, motile by means of several polar flagella. Poly-β-hydroxybutyrate is accumulated. Colonies on 2% glucose–peptone agar are mucoid and similar to *H. seropedicae* when grown on JNFb agar plates. Oxidase positive. No hydrolysis of gelatin, starch, or Tween 80. Denitrification is negative, although most strains reduce nitrate to nitrite. Optimal pH and temperature for N₂-dependent growth in semisolid JNFb medium are 5.8 and 30°C, respectively, although growth can occur at temperatures as high as 40°C. N₂ is fixed as efficiently as by *H. seropedicae*. The favored carbon sources are those described for *H. seropedicae*. Growth and N₂ fixation occur in the presence of 10% sucrose, although this carbon source is not used (osmotolerance effect). The strains also do not use maltose, cellobiose, raffinose, salicin, and *meso*-inositol. The ability to tolerate high concentrations of these carbon sources was not tested. Sensitive to kanamycin, spectinomycin, and streptomycin and resistant to nalidixic acids and low concentrations of novobiocin and rifampicin.

Occurrence is apparently limited to sugar cane. Causes mottled stripe disease, mainly on sugar cane genotypes from regions in which high-nitrogen fertilizer applications are used. In addition, the organism can cause red stripe disease when artificially inoculated by injection into leaves of *Sorghum vulgare* and *Pennisetum purpureum*.

The mol% G + C of the DNA is: 62–63 (T_m).

Type strain: ATCC 19308, BR11192, DSM 9440, LMG 2286, NCPPB 1027.

GenBank accession number (16S rRNA): AB021424.

Other Organisms

A group, also called EF group 1, comprising two subgroups, 1a (8 strains) and 1b (14 isolates), and showing many physiological and genetics characteristics resembling *Herbaspirillum* was identified during the description of the *H. rubrisubalbicans* species. Subgroup 1a is differentiated from subgroup 1b by its ability to use *meso*-erythritol and benzoate as sole carbon sources. $T_{m(e)}$ (°C) values from the DNA–rRNA hybrids formed by the DNA hybridization of strains from this EF1 group with the 23S rRNA from *H. rubrisubalbicans* strain LMG 2286 vary from 77.0–79.3. These values are very close to those observed when strains of *H. seropedicae* were used (77.1–78.3). Because of the high level of DNA relatedness to the nitrogen-fixing species, this group has been tentatively included in the genus *Herbaspirillum* and has been provisionally called species 3. However, the correct taxonomic position of this group will be published elsewhere (Gillis, personal communication). This species has also been separated from other species based on its antigenic properties (Falsen, 1996). All of the strains are of clinical origin (i.e., wound, urine, otitis, gastric juice, respiratory tract, eye secretion, etc.) except for isolates LMG 2285, LMG 6421, and LMG 6416, which were isolated from sugarcane, sorghum, and maize plants, respectively, and previously described as *Pseudomonas rubrisubalbicans*. None of the strains fix nitrogen. The mol% G + C of the DNA of strain LMG 5523 is 61, as determined by the thermal denaturation method (De Ley and van Muylen, 1963) and calculated by the equation of Marmur and Doty (1962), modified by De Ley et al. (1970a).

Genus IV. **Janthinobacterium** De Ley, Segers and Gillis 1978, 164,^AL emend. Lincoln, Fermor and Tindall 1999, 1586

MONIQUE GILLIS AND NIALL A. LOGAN

Jan.thin.o.bac.te′ ri.um. L. adj. *janthinus* violet-colored; Gr. n. *bakterion* a small rod; M.L. neut. n. *Janthinobacterium* a small, violet-colored rod.

Round-ended, straight rods, sometimes slightly curved, 0.8–1.5 × 1.8–6.0 μm, occurring singly and sometimes in pairs or short chains. Definite capsules are not evident, but intercellular slime may be produced. No resting stages are known. Gram negative, occasionally showing bipolar or barred staining and lipid inclusions. **Motile. Strict aerobes. Growth occurs from 4°C to about 30°C, with optimum growth at around 25°C.** Optimal pH 7–8, with no growth below pH 5. **Many strains produce the violet pigment violacein,** but strains producing partly pigmented or nonpigmented colonies are often encountered. Colonies on routine solid media are low convex and round. **Aerobic, having a strictly respiratory metabolism with oxygen as the terminal electron acceptor. Oxidase and catalase positive.** Chemoorganotrophs. Growth occurs on ordinary peptone media. Citrate and ammonium ions can be used as sole carbon and nitrogen sources, respectively. Small amounts of acid, but no gas, are produced

from glucose and some other carbohydrates. **The genus is characterized by the following major phospholipids**: phosphatidyl ethanolamine, phosphatidylglycerol, and diphosphatidylglycerol as polar lipids. **The major fatty acids** are $C_{16:0}$, $C_{16:1\ \omega7c}$, and $C_{17:0\ cyclo}$. Only $C_{10:0\ 3OH}$ and $C_{12:0\ 2OH}$ are synthesized; the $C_{10:0\ 3OH}$ are ester- and (presumably) amide-linked, and the $C_{12:0\ 2OH}$ fatty acids are (presumptively) ester-linked. Q-8 is the major respiratory lipoquinone. Occur in soil and water and are common in temperate climates and cold climates, but can also be isolated from diseased mushroom tissue.

The mol% G + C of the DNA is: 61–67.

Type species: **Janthinobacterium lividum** (Eisenberg 1891) De Ley, Segers and Gillis 1978, 164 (*Chromobacterium lividum* Bergey, Harrison, Breed, Hammer and Huntoon 1923a, 119; *Bacillus lividus* Eisenberg 1891, 81.)

FURTHER DESCRIPTIVE INFORMATION

Pigmentation and colony characteristics The genus *Janthinobacterium* was created to contain the former oxidative, psychrotrophic, violacein-producing species *Chromobacterium lividum* (Sneath, 1984b) and contained solely that species for several years. A second species—*Janthinobacterium agaricidamnosum*—was proposed by Lincoln et al. (1999) based on the results of a polyphasic study on a group of isolates that provoked soft rot on mushrooms. The new species does not form a violet pigment, although in older cultures, a buff, nonfluorescent pigment is seen; the colonies are also slightly mucoid. The texture is more viscous than for the species *J. lividum.* Consequently, the description of the genus has been emended.

The pigment violacein is produced by *J. lividum* on or in media containing tryptophan. It is soluble in ethanol, but not in water or chloroform, and is readily identified by spectrophotometry and by testing with routine reagents (see "Procedures for Testing for Special Characters" in the chapter *Chromobacterium*). Subcultures of pigmented strains may contain partially or completely nonpigmented colonies. A nonpigmented strain is most readily recognized by a colony morphology similar to that of a pigmented strain isolated at the same time under the same conditions. Once it has been confirmed as an oxidase- and catalase-positive, Gram-negative rod, such an isolate should be stained to determine the type of flagellation. If it possesses the characteristic polar and lateral flagella, it should then be subjected to tests that differentiate the four genera *Chromobacterium, Iodobacter, Janthinobacterium,* and *Vogesella*—all of which belong to the class *Betaproteobacteria* and can produce a violet pigment (Table BXII.β.22). Although young cultures of *Aeromonas, Pseudomonas,* and *Vibrio* spp. grown on solid media sometimes produce lateral flagella in addition to their usual polar flagella, they give patterns of results that differ from those for the violet-pigmented organisms in the differential tests.

The characteristic flagellar arrangement in *J. lividum* is best seen with cells from young cultures on solid media. The single polar flagellum is inserted at the tip of the cell, shows long, shallow waves, and often stains faintly. The lateral flagella are usually long and 1–4 in number, although up to eight may occur. They may be inserted subpolarly or laterally, usually show deep, short waves, and stain readily (see the chapter on *Iodobacter*, Fig. BXII.β.71).

Atypical strains of *J. lividum*, characterized by gelatinous, tough colonies and differing from typical *J. lividum* in several biochemical test reactions (Tables BXII.β.22 and BXII.β.24), represented 16% of the *J. lividum* isolates in the studies of Moss and Ryall (1981) and Logan (1989). Atypical strains may prove difficult to subculture or maintain in the laboratory, and until further strains are isolated and studied, *J. lividum* must be regarded as a heterogeneous species. However, Sneath (1984b) has found that the strains with gelatinous colonies (atypical strains) cross-reacted serologically with other *J. lividum* strains and do not notably differ from them in other ways. The similarity between *J. agaricidamnosum* and the atypical *J. lividum* strains has not been studied.

The second species, *J. agaricidamnosum*, causes soft rot disease on *Agaricus bisporus*, the cultivated mushroom. The cells are motile, but the type of flagellation has not been determined. *J. lividum* strains either cause only a slight necrosis on mushroom tissue blocks or have no effect. *J. agaricidamnosum* and representative strains of *J. lividum* have been studied intensively by Lincoln et al. (1999), and chemotaxonomic and phenotypic characteristics have been found to distinguish both species. Unfortunately, the atypical *J. lividum* strains were not included in this study, and thus it is not possible to decide if some of the atypical strains belong in *J. agaricidamnosum*.

Chemotaxonomic features Whole-cell fatty acid analyses (Moss et al., 1980) have been performed on both species and on their phylogenetic neighbors. Special methods were used, which allowed the selective hydrolysis of ester- and amide-linked fatty acids (B. Tindall, personal communication). The respiratory lipoquinones and polar lipids have also been studied (Lincoln et al., 1999).

Sensitivity to antibiotics Members of *J. lividum* are sensitive to a number of antibiotics (Table BXII.β.24). *J. agaricidamnosum* strains are sensitive to erythromycin (15 μg/disc), streptomycin (10 μg/ disc), tetracycline (30 μg/disc), and nalidixic acid (30 μg/disc).

ENRICHMENT AND ISOLATION PROCEDURES

Isolation of *J. lividum* from soil may be accomplished by the method of Corpe (1951). A few crumbs of soil are placed in a Petri dish and 10–25 ml of sterile water is added. About 5–6 grains of heat-sterilized, polished rice are added, and the plates are closed and incubated at 20°C for several weeks. Incubation at 4-10°C may be advantageous. Strains can be isolated by plating from violet patches on the rice onto nutrient agar and incubating at 20°C. Instead of nutrient agar, 0.025% yeast extract in water-agar or a medium consisting of 1% mashed and strained boiled rice in 1.5% water-agar supplemented with L-tryptophan (25 mg/l) may be used to improve pigmentation. *J. lividum* can also be enriched and isolated on the same media as given in detail for *Chromobacterium* (see chapter on *Chromobacterium*). According to Moss and Ryall (1981), *Chromobacterium* spp. grow rapidly and can be selected on a citrate ammonium salt agar medium[1]. Koburger and May (1982) have recovered *J. lividum* from a variety of food samples only on Bennett's agar[2] at 25°C. They found higher counts in water and soil samples on this medium than on the medium of Ryall and Moss (1975) or on *Aeromonas* agar (Rippey and Cabelli, 1979). Maximal numbers of

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1. Citrate ammonium salt agar is composed of (g/l): NaCl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; $NH_4H_2PO_4$, 1.0; K_2HPO_4, 1.0; Ionoagar no. 2 (Oxoid), 15.

2. Bennett's agar consists of (g/l): tryptose, 5; trehalose, 5; yeast extract, 2; NaCl, 3; KCl, 2; $MgSO_4 \cdot 7H_2O$, 0.2; $FeCl_3 \cdot 6H_2O$, 0.1; bromothymol blue, 0.04.

TABLE BXII.β.22. Patterns of results in tests useful for differentiating typical and atypical strains of *Janthinobacterium lividum* from *Chromobacterium*, *Iodobacter*, and *Vogesella* species[a,b]

Characteristic	*J. lividum* (typical)	*J. lividum* (atypical)	*Iodobacter fluviatilis*	*Vogesella*	*C. violaceum*
Number of strains tested	68	14	53	17	9
Pigment produced:					
Indigoidine				+	
Violacein	+	+	+		+
Fluorescence[c]	−[d]			+ (100)	−[d]
Colonies on 1/4 nutrient agar:					
Spreading	− (0)	− (0)	d (83)	nr	− (0)
Gelatinous	d (36)	+ (100)	− (4)	nr	− (0)
Tough	− (7)	d (71)	− (0)	nr	− (0)
Growth at:					
4°C	+ (94)	d (87)	+ (100)	+ (100)	− (0)
37°C	− (0)	− (0)	− (0)	+ (100)	+ (100)
Anaerobic growth	− (3)	− (0)	+ (100)	nr	+ (100)
Nitrate reduction	d (84)	nr	+ (98)	+ (100)	d (87)
Production of indole	− (0)	− (0)	− (0)	+ (100)	− (0)
Hydrolysis of Tween 80	+[d]	nr	nr	− (0)	+[d]
Growth on citrate	+ (95)	+ (94)	d (85)	− (0)	+ (100)
Glucose:					
Fermented	− (0)	− (0)[e]	+ (100)	− (0)	− (0)[f]
Oxidized	+ (97)	d (57)[e]	− (0)	− (0)	− (0)[f]
Acid from:					
L-Arabinose [g]	+ (100)	+ (87)	− (0)	− (0)	− (0)
D-Maltose	+ (98)	+ (94)	+ (100)	nr	− (0)
myo-Inositol [h]	+ (100)	− (0)	− (0)	d (35)	− (0)
Trehalose	− (1)	d (87)	+ (100)	nr	+ (100)
Gelatinase	+ (100)	d (69)	+ (100)	− (0)	+ (100)
Lactate utilization	+ (100)	d (75)	− (0)	nr	+ (100)
Esculin hydrolysis	+ (100)	− (0)	− (0)	nr	− (0)
Arginine hydrolysis	− (0)	− (0)	− (0)	nr	+ (100)
Chitin digestion	− (5)	nr	nr	− (0)	+ (100)[i]
Caseinase	− (5)	− (0)	+ (90)	− (0)	+ (100)
HCN production	− (0)	− (0)	− (0)	nr	+ (100)

[a]Symbols: see standard definitions; nr, data not reported. Numbers in parentheses indicate the % of strains giving a positive reaction.

[b]Data from Logan (1989) with later amendments.

[c]Fluorescence on chalk agar (Starr et al., 1960) is determined with short wavelength illumination. All *Vogesella* strains exhibit very weak fluorescence.

[d]Only tested for the type strain.

[e]Some strains give no reaction in this test.

[f]Occasional strains are oxidative.

[g]Similar patterns of results are obtained with D-cellobiose and D-galactose.

[h]Similar patterns of results are obtained with D-sorbitol.

[i]Negative for the type strain (Grimes et al., 1997).

J. lividum have been isolated at 25°C from turbot gills (Muddaris and Austin, 1988) on a casein–tryptone medium.[3]

J. agaricidamnosum can be isolated by transferring samples of diseased mushroom tissue to sterile 0.25 × Ringer's solution and inoculating 10-fold dilution series onto *Pseudomonas* agar F (PAF, Merck) and nutrient agar, with incubation at 25°C for 24 h. Single colonies are isolated from the dilution plates and cultured at 25°C on the appropriate media. Each isolate is first "white line tested" for identity as *P. tolaasii* (Wong and Preece, 1979), a common pathogen for mushrooms, and then tested on mushroom blocks in an initial pathogenicity test (Gandy, 1968). The isolates are plated on *Pseudomonas* isolation agar (PIA, Difco). The cultures are reported to die if left more than 10 d without subculturing.

J. lividum strains have been isolated and identified in microbial mats in Antarctica (Brambilla et al., 2001) and in wet silk thread that became violet in color (Shirata et al., 2000). The question

has arisen as to whether violacein can be used to dye natural and synthetic fibers.

J. lividum JAC1 has also been described as producing metallo-β-lactamase upon exposure to β-lactams. The β-lactamase determinant encodes for a new member of the highly divergent subclass B3 lineage of metallo-β-lactamases (Rossolini et al., 2001).

MAINTENANCE PROCEDURES

Janthinobacterium lividum strains may survive for several years in dilute peptone water (0.1% peptone) at room temperature. They can also be preserved indefinitely by lyophilization or by freezing in nutrient broth containing 15% glycerol. Strains of *J. agaricidamnosum* can be stored freeze-dried or in liquid nitrogen. Working cultures must be subcultured weekly.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Methods for demonstrating special features in *J. lividum* (production of violacein, flagellar arrangement, growth temperature, oxidase reaction, oxidative attack of glucose, aerobic growth, acid production from carbohydrates, esculin hydrolysis, and lactate

3. Casein–tryptone medium (g/l): CaCl₂, 1.0; yeast extract, 1.0; beef extract, 5.0; casein, 6.0; tryptone, 2.0; agar no. 1, 15.0; aged sea water, 750 ml; pH 7.2.

TABLE BXII.β.23. Differential characteristics of three typical *J. lividum* strains and all strains of *J. agaricidamnosum* based on respiratory activity using Biolog GN plates[a]

Biolog GN test[b,c]	*J. lividum*	*J. agaricidamnosum*
Gentiobiose	−	d
Lactulose	−	d
D-Trehalose	−	+
Turanose	−	d
D-Glucuronic acid	−	d
m-Inositol	−	d
Urocanic acid	−	d
Dextrin	+	−
N-Acetyl-D-galactosamine	+	−
D-Fucose	+	d
Maltose	+	−
D-Mannose	+	−
D-Sorbitol	+	d
Xylitol	+	−
DL-Lactic acid	+	−
Propionic acid	+	d
Succinic acid	+	−
Succinamic acid	+	−
D-Alanine	+	−
L-Alanine	+	−
L-Asparagine	+	−
L-Aspartic acid	+	−
L-Glutamic acid	+	−
L-Proline	+	−
Inosine	+	−
Tween 40	d	+
Tween 80	d	+
L-Arabinose	d	−
L-Fucose	d	−
D-Galactose	d	−
D-Mannitol	d	−
D-Psicose	d	−
D-Raffinose	d	−
L-Rhamnose	d	−
Acetic acid	d	−
p-Hydroxyphenylacetic acid	d	d
α-Ketobutyric acid	d	−
α-Ketoglutaric acid	d	+
α-Ketovaleric acid	d	−
Malonic acid	d	+
D-Serine	d	−
L-Serine	d	−
L-Threonine	d	−
Glycerol	d	+

[a]Symbols: see standard definitions.

[b]The following tests are negative for all strains: α-xyclodextrin, glycogen, *N*-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, *i*-erythritol, α-D-lactose, D-melibiose, methyl-β-D-glucoside, monomethylsuccinate, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, DL-carnitine, γ-aminobutyric acid, uridine, thymidine, phenylethylamine, putrescine, 2-amino-ethanol, and 2,3-butanediol.

[c]The following tests are positive for all strains: α-D-glucose, sucrose, methylpyruvate, formic acid, β-hydroxybutyric acid, bromosuccinic acid, alaninamide, L-alanylglycine, glycyl-L-aspartic acid, glycyl-L-glutamic acid, DL-glycerol phosphate, glucose-1-phosphate, and glucose-6-phosphate.

utilization) are described in the chapter on the genus *Chromobacterium.*

Janthinobacterium agaricidamnosum can be tested for pathogenicity on mushroom tissue blocks or on growing mushrooms. In the latter experiments, varying percentages of relative humidity (85–95%) have been applied, leading to the conclusion that there is a general trend in which no disease symptoms appear at below 71.5% relative humidity (Lincoln et al., 1999).

A special technique has been used by Lincoln et al. (1999) to study the release of ester-linked or amide-linked fatty acids.

DIFFERENTIATION OF THE GENUS *JANTHINOBACTERIUM* FROM OTHER GENERA

Table BXII.β.22 presents characteristics differentiating typical and atypical strains of *Janthinobacterium* from the other violet-pigment-producing genera of the class *Betaproteobacteria*—i.e., *Chromobacterium*, *Iodobacter*, and *Vogesella*. The percentage fatty acid composition and some phenotypic characteristics that differentiate the genus *Janthinobacterium* from *Duganella*, *Herbaspirillum*, *Oxalobacter*, *Massilia*, and *Paucimonas* are listed in Table BXII.β.25.

Janthinobacterium is phylogenetically more closely related to the following organisms: *Duganella*, *Herbaspirillum*, *Telluria*, *Oxalobacter*, *Massilia*, and to *Pseudomonas lemoignei*, for which a new genus, *Paucimonas*, has recently been proposed (Jendrossek, 2001). *Oxalobacter* can be differentiated from the other genera by the lower mol% G + C of its DNA (48–51), its obligate anaerobic growth requirement, and its strict use of only oxalate and oxamate as sole sources of carbon for growth. *Telluria* can be differentiated from *Janthinobacterium*, *Duganella*, *Paucimonas*, and *Herbaspirillum* by its relatively high mol% G + C (67–72) and its ability to grow on starch and glycogen. Chemotaxonomically, the genera *Janthinobacterium*, *Paucimonas*, *Duganella*, *Massilia*, and *Herbaspirillum* can be differentiated from each other by their quantitative fatty acid compositions (See Table BXII.β.25). Moreover, *Duganella* is the only member of this group to exhibit flocculent growth; *Massilia* has a tendency to form flocs.

TAXONOMIC COMMENTS

Sneath (1984b) described the genus *Chromobacterium* as containing two species: *C. violaceum* and *C. lividum*. The former species was fermentative and mesophilic, growing at 37°C, but not at 4°C, whereas the latter species was nonfermentative and psychrotrophic, with growth occurring at 4°C, but not at 37°C. Both species were described based on the numerical analysis of their phenotypic features. Sneath stressed that both groups were phenotypically very different from each other, but kept them in one genus because, at that time, no genomic results were available. Later genomic studies indicated that the two species differ too much to be contained in one genus, and the new genus *Janthinobacterium* was proposed for the former *Chromobacterium lividum* (De Ley et al., 1978; Sneath, 1984b). The phylogenetic affiliation was determined first by rRNA–DNA hybridization (De Ley et al., 1978) and later by 16S rDNA sequence analysis (Lincoln et al., 1999). *Janthinobacterium* is a member of the class *Betaproteobacteria*, in which it constitutes a cluster together with the genera *Herbaspirillum*, (Baldani et al., 1986a, 1996; Kirchhof et al., 2001) *Duganella* (Hiraishi et al., 1997b), *Oxalobacter* (Allison et al., 1985a; Dehning and Schink, 1989b), *Telluria* (Bowman et al., 1993b), *Massilia* (La Scola et al., 1998a), and *Paucimonas* (Jendrossek, 2001). The highest 16S rDNA sequence similarity is found among *Janthinobacterium*, *Herbaspirillum*, and *Duganella* (95–96.4%), raising the question as to whether the latter genera might be merged. Baldani et al. (1996) considered the single violacein-producing species *J. lividum* to be phenotypically different enough from *Herbaspirillum* to remain in a separate genus. With the inclusion of a second species in *Janthinobacterium*, Lincoln et al. (1999) raised this question again and performed a broad chemotaxonomic study within this rRNA cluster. The results obtained indicated clearly that the genera *Herbaspirillum*,

TABLE BXII.β.24. Characteristics of typical and atypical strains of *Janthinobacterium lividum* and of *Janthinobacterium agaricidamnosum*[a]

Characteristic	*J. lividum* (typical)	*J. lividum* (atypical)	*J. agaricidamnosum*
Formation of cell chains	− (9)	d (81)	nr
Colonies on 1/4 × nutrient agar:			
Spreading	−	−	nr
Gelatinous	d (36)	d (61)	nr
Pigmented	d (78)	d (50)	nr
Zoning of pigment	d (58)	d (61)	nr
Growth on:			
Minimal medium	+ (100)	− (7)	nr
1/4 × NA	d (47)	d (62)	nr
Growth at:			
4°C	+ (100)	d (87)	nr
30°C	d (87)	d (81)	+ (100)
37°C	− (0)	− (0)	+ (100)
Growth in the presence of:			
1% NaCl	+ (98)	d (19)	+ (100)
2% NaCl	d (28)	− (6)	+ (100)
4% NaCl	− (0)	− (0)	− (0)
Growth at:			
pH 4	d (22)	− (6)	+ (100)
pH 5	+ (98)	d (87)	+ (100)
Acid from:			
ʟ-Arabinose	+ (100)	d (87)	nr
ᴅ-Cellobiose	+ (100)	+ (94)	nr
ᴅ-Fructose	+ (100)	d (12)	nr
ᴅ-Galactose	+ (100)	d (87)	nr
Gluconate	− (2)	− (0)	nr
ᴅ-Glucose	+ (100)	d (75)	+ (100)
Glycerol	+ (98)	− (0)	nr
Glycogen	− (2)	d (81)	nr
myo-Inositol	+ (100)	− (0)	nr
Inulin	d (52)	d (37)	nr
Lactose	d (55)	d (81)	nr
ᴅ-Maltose	+ (98)	+ (94)	nr
ᴅ-Mannitol	+ (100)	d (62)	nr
ᴅ-Mannose	+ (97)	d (87)	nr
Melezitose	− (0)	− (0)	nr
N-Acetylglucosamine	− (2)	− (0)	nr
ᴅ-Raffinose	− or w (30)	− (0)	nr
ᴅ-Sorbitol	+ (95)	− (0)	nr
Starch	− (0)	− or w (6)	nr
Sucrose	+ (92)	d (87)	nr
Trehalose	− (2)	d (87)	nr
ᴅ-Xylose	+ (100)	d (69)	nr
Catalase, oxidase	+ (100)	+ (100)	+ (100)
Nitrate reduction	d (84)	d (81)	− (0)
Nitrite reduction	d (50)	d (25)	− (0)
Production of HCN	− (0)	− (0)	nr
Egg yolk reaction	− (0)	− (0)	nr
Hemolysis	+ (90)	d (19)	nr
Carbon sources:			
Acetate	+ (100)	d (69)	nr
Citrate	+ (95)	+ (94)	d
Fumarate	+ (100)	+ (94)	nr
Glycerate	d (74)	+ (100)	nr
Lactate	+ (100)	d (75)	nr
Malate	+ (100)	+ (94)	nr
Propionate	+ (100)	d (12)	nr
Pyruvate	+ (100)	+ (100)	nr
Succinate	+ (100)	+ (94)	nr
Tartrate	− (0)	− (0)	nr
Hydrolysis of:			
Arginine	− (0)	− (0)	− (0)
Casein	− (5)	− (0)	nr
Esculin	+ (100)	− (0)	− (0)
Gelatin	+ (100)	d (69)	− (0)
Starch	− (0)	− (0)	nr
Resistant to:			
Ampicillin, 25 µg/disk	d (82)	d (80)	nr
Cephaloridine, 25 µg/disk	d (76)	d (75)	nr
Colistin sulfate, 10 µg/disk	+ (100)	+ (100)	nr

(*continued*)

TABLE BXII.β.24. *(cont.)*

Characteristic	*J. lividum* (typical)	*J. lividum* (atypical)	*J. agaricidamnosum*
Chloramphenicol, 10 μg/disk	d (57)	d (50)	nr
Chlortetracycline, 10 μg/disk	− (6)	− (0)	nr
Furazolidone, 50 μg/disk	d (27)	d (31)	nr
Kanamycin, 30 μg/disk	− (0)	− (0)	nr
Neomycin, 10 μg/disk	− (0)	d (12)	nr
Nalidixic acid, 30 μg/disk	d (12)	d (12)	− (0)
Nitrofurantoin, 200 μg/disk	+ (100)	+ (100)	nr
Oxytetracycline, 10 μg/disk	− (0)	d (18)	nr
Penicillin G, 1.5 IU	+ (100)	+ (94)	nr
Streptomycin, 10 μg/disk	− (0)	− (0)	− (0)
Sulfafurazole, 100 μg/disk	d (60)	− (0)	nr
Sulfafurazole, 500 μg/disk	− (0)	− (0)	nr
Vibriostatic agent O/129, 50 μg/disk	+ (100)	+ (100)	nr

[a]Symbols: see standard definitions; nr, data not reported. Numbers in parentheses indicate the % of strains giving a positive reaction.

Duganella, Oxalobacter, and *Paucimonas* share several chemotaxonomic features, but that the genus *Janthinobacterium* has peculiar features that distinguish it from its neighbors and thus deserve to be maintained in a separate genus. Moreover, Lincoln et al. (1999) have shown that isolates from diseased mushrooms share 99% 16S rDNA sequence similarity with *Janthinobacterium lividum,* thereby leading to the inclusion of this non-violacein-producing group in the genus *Janthinobacterium.* The phenotypic (auxanographic) results, DNA–DNA hybridizations (only 35% DNA–DNA hybridization has been reported between the type strains of both species), and chemotaxonomic results (cyclo-fatty acids and polar lipids) have shown that these mushroom isolates belong in a separate species, for which the name *J. agaricidamnosum* was proposed. Consequently, the genus *Janthinobacterium* has been emended to contain these mushroom isolates as a new species.

The 16S rDNA similarity between *Janthinobacterium, Duganella, Herbaspirillum, Paucimonas, Telluria, Massilia,* and *Oxalobacter* ranges from 92.7–96.4%.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *JANTHINOBACTERIUM*

The phenotypic and chemotaxonomic features that differentiate *J. lividum* and *J. agaricidamnosum* are given in Table BXII.β.24. Differential tests using the commercial Biolog GN and the API 50CH systems are listed in Tables BXII.β.23 and BXII.β.26.

List of species of the genus Janthinobacterium

1. **Janthinobacterium lividum** (Eisenberg 1891) De Ley, Segers and Gillis 1978, 164[AL] (*Chromobacterium lividum* Bergey, Harrison, Breed, Hammer and Huntoon 1923a, 119; *Bacillus lividus* Eisenberg 1891, 81.)

li'vi.dum. L. adj. *lividum* leaden-colored, dark blue.

The description of *J. lividum* is based on the studies of Leifson (1956), Sneath (1956, 1960), De Ley et al. (1978), and Logan (1989). The characteristics are as described for the genus and in Tables BXII.β.22, BXII.β.23, BXII.β.24, BXII.β.25, and BXII.β.26, with the following additional features. Pigmentation may be produced in concentric rings or in sectors within colonies, and subcultures may contain unpigmented colonies. Colonies may be butyrous, gelatinous, or rubbery or may comprise a gelatinous outer layer with butyrous or mucoid growth beneath. Growth in nutrient broth shows a violet ring at the junction of the liquid surface and the wall of the vessel; strains forming gelatinous or rubbery colonies may form a tough, violet pellicle in broth cultures.

Characteristics differentiating typical strains from atypical strains are listed in Tables BXII.β.22 and BXII.β.24.

Motile by means of a single polar flagellum and usually one or more subpolar or lateral flagella. Indole negative, Voges–Proskauer negative. Nitrate and nitrite are reduced, sometimes with visible gas production. Phosphatase positive. Arylsulfatase negative. Other characteristics are shown in Tables BXII.β.23, BXII.β.24, and BXII.β.25. Resistant to benzylpenicillin (10 μg/ml) and O/129 (2,4-diamino-6,7-diisopropyl pteridine) by disc diffusion method, (50 μg/disc).

Janthinobacterium lividum occurs in soil and water and is common in temperate and cold climates. It occasionally causes food spoilage, and these strains may produce active metalloproteinases (Dainty et al., 1978).

The mol% G + C of the DNA is: 61–67 (T_m).

Type strain: H-24, ATCC 12473, CCM 160, DSM 1522, NCIB 9130, NCTC 9796.

GenBank accession number (16S rRNA): Y08846.

2. **Janthinobacterium agaricidamnosum** Lincoln, Fermor and Tindall 1999, 1577[VP]

a.ga'ri.ci.dam.no.sum. L. masc. n. *agaricus* mushroom; L. adj. *damnosum* damaging; *agaricidamnosum* damaging mushroom.

The description of *J. agaricidamnosum* is based on the studies of Lincoln et al. (1999). The characteristics are as described for the genus and in Tables BXII.β.23, BXII.β.24, BXII.β.25, and BXII.β.26, with the following additional features. Low, convex, round, beige colonies are formed on solid media. Maximum growth temperature, 30°C; minimum, 2°C. No growth occurs below pH 5 or in media containing 2.9% NaCl. Acid is produced from glucose. Indole and Voges–Proskauer negative. Nitrate and nitrite are not reduced. No violet pigment is produced. Resistant to penicillin G (10 μg/disc) and vancomycin (30 μg/disc). The chemical composition is as described for the genus.

The type strain was isolated as the cause of soft rot disease of the cultivated mushroom *A. bisporus.*

The mol% G + C of the DNA is: 64.2 (T_m).

Type strain: W1r3, DSM 9628, NCPPB 3945.

GenBank accession number (16S rRNA): Y08845.

TABLE BXII.β.25. Percentage fatty acid composition and some phenotypic characteristics to differentiate members of the genera *Janthinobacterium*, *Duganella*, *Herbaspirillum*, *Oxalobacter*, *Massilia*, and *Paucimonas*[a]

Characteristic	*Janthinobacterium*[b]	*Duganella*[c]	*Paucimonas*[d]	*Herbaspirillum*[e]	*Oxalobacter*[f]	*Telluria*[g]	*Massilia*[h]
Fatty acid, % of total:							
$C_{10:0}$	0	2	0	0.0–0.9	0	nr	0.68
$C_{10:0\ 3OH}$	3.1–4.0	+ (89)[i]	3.6	3.1–4.0	0	nr	5.66
$C_{12:0}$	3.6–5.2	4	5.3	0.0–4.1	0-1	nr	6.49
$C_{12:0\ 2OH}$	TR-0.94	0	0	0.0–1.1	0	nr	2.77
$C_{12:0\ 3OH}$	0	0	5.7	2.6–3.7	4-6	nr	
$C_{14:0}$	1.0–1.4	3	1.3	0.0–3.5	1-2	nr	3.3
$C_{14:0\ 2OH}$	0	0	4.7	1.0–2.8	8-10	nr	nr
$C_{16:1\ \omega7c}$	22.4–40.7	41[j]	39.3	32.6–39.7	nr	nr	48.3
$C_{16:1}$		0	0	1.9–3.8	Tr-1[j]	nr	nr
$C_{16:0}$	35.3–40.9	38	12.8	19.8–23.7	28-33	nr	21.3
$C_{17:0\ cyclo}$	9.8–26.7	nr	1.9	2.9–5.9	3-34	nr	nr
$C_{18:1\ \omega7c}$	1.38–3.45	12[j]	25.5	19.4–23.2	nr	nr	nr
$C_{18:1}$	nr	nr	nr	0.8-2.7	Tr-13[j]	nr	8.14
$C_{18:0}$	0	nr	0	nr	Tr-5	nr	nr
$C_{19:0\ cyclo}$	0	nr	0	nr	14–38	nr	nr
Growth on:							
D-Ribose	+	−	−	+	−	−	nr
Glycerol	+	−	−	nr	−	v	nr
D-Mannitol	+	−	−	+	−	v	nr
Lactose	−	+	−	nr	−	v	+
Glucose	+	+	−	+	− [e]	+	−
D-Mannose	+	+	−	+	−	+	+
D-Fructose	+	+	−	+	− [e]	+	−
Sucrose	+	+	−	−	nr	−	−
Malate	+	+	−	+	−	nr	+
Starch	−	−	nr	−	−	+	nr
Glycogen	−	−	nr	−	nr	+	nr
Flocculent growth	−	+ [k]	−	−	−	−	+ [k]
Cell shape:							
Straight rods, sometimes slightly curved	+	−	+	−	+	−	nr
Straight rods only	−	+	−	−	+	+	+
Vibrioid to helical	−	−	−	+	−	−	nr
Hydrolysis of starch	−	+	−	nr	nr	+	+
Hydrolysis of gelatin	+	+	−	nr	− [l]	+	+
Mol% G + C of the DNA	61–67	63–64	57–61	60–65	48–51	67–72	64.6

[a]Symbols: see standard definitions; Tr, trace; nr, data not reported,

[b]Data from Lincoln et al. (1999) and from Table BXII.β.22.

[c]Data from Hiraishi et al. (1997b).

[d]Data from Delafield et al. (1965), Mergaert et al. (1996), and Jendrossek (2001).

[e]Data from Baldani et al. (1986a, 1996), and Lincoln et al. (1999).

[f]Data from Allison et al.(1985a), Dawson et al. (1980b), Dehning and Schink (1989b), and Lincoln et al. (1999).

[g]Data from Bowman et al. (1993b).

[h]Data from La Scola et al. (1998a).

[i]Reported as % of total 3-OH component by Hiraishi et al. (1997b).

[j]Hiraishi et al. (1997b) and Allison et al. (1985a) do not distinguish which isomers of 16:0 and 18:1 are present.

[k]Tendency to form flocs.

[l]Available only for *O. formigenes*.

TABLE BXII.β.26. Differential characteristics of three typical *J. lividum* strains and all *J. agaricidamnosum* strains based on carbohydrate utilization in API 50CH[a]

API 50CH test[b,c]	*J. lividum*	*J. agaricidamnosum*
Trehalose	−	+
β-Gentiobiose	−	+
D-Arabinose	+	−
L-Arabinose	+	−
D-Xylose	+	−
Galactose	+	−
Sorbitol	+	−
Arbutin	+	−
Salicin	+	−
Cellobiose	+	−
Maltose	+	−
Xylitol	+	−
D-Lyxose	+	−
L-Fucose	+	−

(*continued*)

TABLE BXII.β.26. (*cont.*)

API 50CH test[b,c]	*J. lividum*	*J. agaricidamnosum*
2-Ketogluconate	+	−
Rhamnose	d	−
N-Acetylglucosamine	d	−
Inulin	d	−
D-Raffinose	d	−
Citrate	+	d
Phenylacetate	+	−
Caprate	d	−
Adipate	d	−

[a]Symbols: see standard definitions.

[b]The following tests are negative for all strains: assimilation of gluconate, erythritol, L-xylose, adonitol, methyl-β-xyloside, L-sorbose, dulcitol, methyl-α-D-mannoside, methyl-α-D-glucoside, amygdalin, esculin, lactose, melibiose, melezitose, amidon, glycogen, D-turanose, D-tagatose, D-fucose, and L-arabitol.

[c]The following tests are positive for all strains: assimilation of glucose, malate, mannose, mannitol, glycerol, ribose, D-fructose, inositol, saccharose, and D-arabitol.

Genus V. **Massilia** La Scola, Birtles, Mallet and Raoult 2000, 423[VP] (Effective publication: La Scola, Birtles, Mallet and Raoult 1998a, 2852)

THE EDITORIAL BOARD*

Mas.sil' i.a. L. n. *Massilia* Latin name of Marseille, France.

Gram-negative nonsporeforming motile rods (1.0 × 3.0 μm). **Strictly aerobic**; catalase positive and oxidase negative. **Grow in 3% NaCl.** No denitrification. Produce arginine dihydrolase and urease. Hydrolyze esculin, gelatin, and starch. **No acid from carbohydrates.** Utilize L-alanine, L-arabinose, D-cellobiose, fumarate, D-galacturonate, gentisate, D-glucuronate, α-ketoglutarate, α-lactose, D-malate, L-malate, malonate, maltose, D-mannose, protochachuate, α-L-rhamnose, succinate, D-tagatose, and D-xylose. Does not utilize D-alanine, L-aspartate, citrate, D-fructose, D-galactose, D-glucose, L-glutamate, DL-glycerate, β-hydroxybutyrate, DL-lactate, D-melezitose, mucate, D-saccharate, L-serine, sucrose, L-tartrate, or D-trehalose. Major fatty acids are $C_{16:1\ \omega 7c}$, $C_{16:0}$, $C_{18:1}$, $C_{12:0}$, $C_{10:0\ 3OH}$, $C_{14:0}$, and $C_{12:0\ 2OH}$.

The mol% G + C of the DNA is: 64 ± 1.8 (HPLC).

Type species: **Massilia timonae** La Scola, Birtles, Mallet and Raoult 2000, 423 (Effective publication: La Scola, Birtles, Mallet and Raoult 1998a, 2852).

FURTHER DESCRIPTIVE INFORMATION

Colonies are straw-colored; flocs or films form in liquid medium; older cultures at 37°C may contain filamentous cells. Growth occurs on MacConkey agar, Columbia agar containing 5% sheep blood, chocolate agar, and trypticase soy agar.

The organism was isolated from the blood of an immunocompromised patient suffering from meningoencephalitis. The infection was viewed as opportunistic because the 16S rDNA sequence obtained from the organism was most similar to those of the soil microorganisms *Telluria mixta*, *Telluria chitinolytica*, and *Duganella zoogloeoides*, which are not known to be pathogenic to humans. Other characteristics, including biochemical test profiles and mol% G + C of the DNA, were consistent with this relationship.

ENRICHMENT AND ISOLATION PROCEDURES

The organism was isolated from blood using an automated blood culture system (BACTEC NR-860).

Editorial Note: The description in this chapter is based on a single strain—the type strain. Lindquist, et al., 2003, J. Clin. Microbiol. *41:* 192–196 reported the isolation of additional strains and proposed an emended species description to include them.

List of species of the genus Massilia

1. **Massilia timonae** La Scola, Birtles, Mallet and Raoult 2000, 423[VP] (Effective publication: La Scola, Birtles, Mallet and Raoult 1998a, 2852)

   ti.mon.ae. L. gen. n. belonging to Timone, because the organism was isolated from a patient at L'Hôpital de la Timone.

   The species description and the genus description are identical.

   The mol% G + C of the DNA is: 64 ± 1.8 (HPLC).

   Type strain: UR/MT95, CIP 105350.

   GenBank accession number (16S rRNA): U54470.

Genus VI. **Telluria** Bowman, Sly, Hayward, Spiegel and Stackebrandt 1993b, 123[VP]

LINDSAY I. SLY AND MARK FEGAN

Tel.lu' ri.a. L. fem. n. *Tellus* a Roman goddess of the earth, also the ground or earth; M.L. fem. n. *Telluria* from the earth.

Cells are **rod-shaped**, 2–3 × 0.5–1.0 μm. **Gram negative**. Filamentous cells up to 30 μm long are formed occasionally in older cultures. **Cells occur singly, in pairs, or in short chains**. **Motile**. Exhibit **mixed flagellation**; cells in liquid media possess a single polar flagellum, while on solid media additional lateral flagella of shorter wavelength occur. **Accumulate poly-β-hydroxybutyrate**. **Strictly aerobic**. **Oxidative** metabolism. **Catalase and oxidase positive**. Surface pellicle formed in static liquid cultures. **Chemoheterotrophic**. Unable to grow chemolithotrophically with hydrogen. **Denitrification does not occur**. **Arginine dihydrolase is absent**. Good growth occurs on media containing carbohydrates and an inorganic or organic combined nitrogen source. Poor growth occurs on media lacking carbohydrates. **NaCl sensitive**; completely inhibited by NaCl concentrations greater than 1.5%, and only poor growth occurs at an NaCl concentration of 0.5%. **Utilize complex polysaccharides**, including starch and xylan. Cellulose is not hydrolyzed. Hydrolyze gelatin, casein, DNA, esculin, and Tween 40, Tween 60, and Tween 80. Produce phosphatase and arylsulfatase. Grow well at temperatures between 20° and 45°C and optimally at 30–35°C; optimal growth occurs at pH 7.0. The **major quinone is ubiquinone 8**. Belongs to the *Betaproteobacteria*. The only known habitat is soil, particularly the rhizosphere.

The mol% G + C of the DNA is: 67–72.

Type species: **Telluria mixta** (Bowman, Sly and Hayward 1989) Bowman, Sly, Hayward, Spiegel and Stackebrandt 1993b, 124 (*Pseudomonas mixta* Bowman, Sly and Hayward 1989, 205.)

FURTHER DESCRIPTIVE INFORMATION

The species of *Telluria* are characterized by their ability to degrade a range of complex carbohydrates. *T. mixta* strains are able to degrade dextran, starch, inulin, pectate, and xylan, but are

unable to degrade cellulose or chitin. A few strains attack alginate and xanthan gum. The first isolate of *T. mixta* (*Pseudomonas* sp. ACM 733, UQM 733, ATCC 49107) was isolated from a sugar cane rhizosphere in Queensland, Australia (Richards and Streamer, 1972). A subsequent taxonomic study of similar dextranolytic strains led to the description of *Pseudomonas mixta* (Bowman et al., 1988, 1989), later transferred to the new genus *Telluria* based on phylogenetic evidence (Bowman et al., 1993b). The dextranases and other carbohydrate-degrading enzymes produced by strain UQM 733 have been studied extensively (Richards and Streamer, 1972; Covacevich and Richards, 1978, 1979). The only known strain of *T. chitinolytica* was isolated from soil in Israel and demonstrated to have bionematicidal activity and potential for the biocontrol of the root-knot nematode *Meloidogyne javinica* (Spiegel et al., 1991).

A distinctive morphological characteristic of the genus is mixed flagellation exhibited when cultures are grown on solid agar media (Bowman et al., 1988). Cells possess a single polar flagellum of long wavelength (2.6 μm) and at least two lateral flagella of shorter wavelength (1.2 μm) (Fig. BXII.β.18). Cells usually possess uniform morphology, but occasionally may be distended by PHB inclusions or form longer cells (Fig. BXII.β.19).
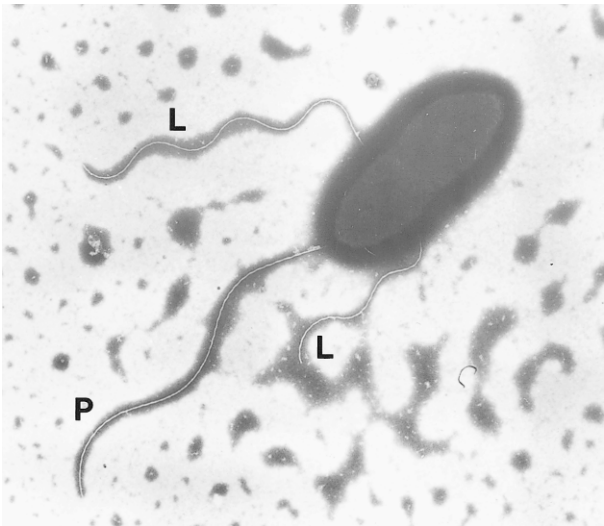
Telluria strains have a predilection for carbohydrates and tricarboxylic cycle intermediates as sources of carbon and energy, although *T. chitinolytica* is slightly less biochemically versatile than *T. mixta*. The spectrum of polysaccharide degradation by *T. chitinolytica* is quite different from that of *T. mixta*, the former able to hydrolyze chitin, but not dextran or pectate (Table BXII.β.27).

Both species utilize D-xylose, L-rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, D-melezitose, maltose, sucrose, cellobiose, trehalose, inulin, starch, xylan, gluconate, 2-ketogluconate, glucuronate, galacturonate, mucate, saccharate, β-hydroxybutyrate, malonate, succinate, DL-lactate, fumarate, DL-malate, pyruvate, L-(+)-tartrate, citrate, D-alanine, L-alanine, L-aspartate, and L-glutamate as sole carbon and energy sources. Unable to utilize D-ribose, acetate, propionate, isobutyrate, valerate, isovalerate, caproate, pelargonate, caprate, oxalate, glutarate, adipate, pimelate, suberate, azelate, sebacate, maleate, D-(−)-tartrate, meso-tartrate, citraconate, itaconate, mesaconate, levulinate, ethanol, propanol, butanol, 1,2-ethanediol, 1,3-propanediol, 2,3-butanediol, adonitol, *meso*-erythritol, *meso*-inositol, sorbitol, anthranilate, *p*-aminobenzoate, benzylamine, sarcosine, betaine, β-ala-

FIGURE BXII.β.18. Electron micrograph of *T. mixta* ACM 1762 showing mixed flagellation. *P*, polar; *L*, lateral flagellum. (Reprinted with permission from J.P. Bowman et al., Systematic and Applied Microbiology *11*: 53–59, 1988, ©Urban & Fischer Verlag GmbH & Co, KG, Jena, Germany.)
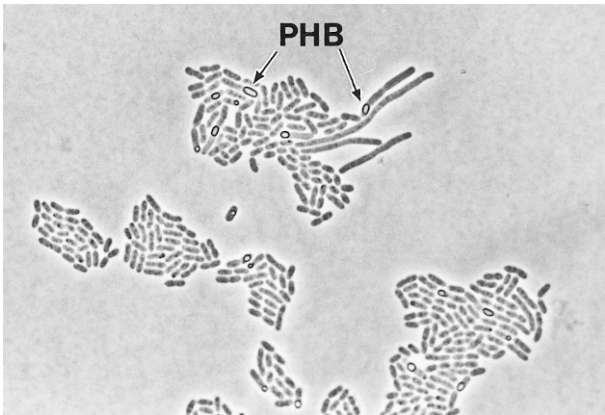
FIGURE BXII.β.19. Microcolony of *T. mixta* ACM 1762. PHB, poly-β-hydroxybutyrate granules. (Reprinted with permission from J. P. Bowman et al., Systematic and Applied Microbiology *11*: 53-59, 1988, ©Urban & Fischer Verlag GmbH & Co, KG, Jena, Germany.)

TABLE BXII.β.27. Differential and descriptive characteristics of the species of *Telluria*[a]

Characteristic	*T. mixta*	*T. chitinolytica*
Yellow pigmentation	−	+
Growth at 45°C	−	+
Nitrate reduced to nitrite	d	−
Urease and β-galactosidase activities	+	−
Lecithinase activity (egg yolk)	−	+
Hydrolysis of:		
Chitin and tributyrin	−	+
Dextran, pectate (pH 5 to 8.3), and Tween 20	+	−
Utilization of:		
DL-Arabinose, lactose, dextran, butyrate, DL-glycerate, glycolate, benzoate, *p*-hydroxybenzoate, DL-serine, DL-threonine, L-arginine, L-citrulline, L-ornithine, and L-phenylalanine	+	−
Glycerol and acetamide	−	+
L-Tyrosine, poly-β-hydroxybutyrate, heptanoate, caprylate, mannitol, phenol, *m*-hydroxybenzoate, benzoyl-formate, DL-mandelate, and quinate	d	−
L-Histidine	d	+

[a]Data from Bowman et al. (1988).

nine, DL-2-aminobutyrate, DL-4-aminobutyrate, DL-2-aminovalerate, DL-5-aminovalerate, L-valine, L-proline, L-hydroxyproline, L-glutamine, L-lysine, L-tryptophan, L-methionine, L-cystein, putrescine, spermine, ethanolamine, histamine, α-amylamine, tryptamine, and pantothenate (Bowman et al., 1993b).

Other biochemical differences include a lack of urease and β-galactosidase activities in *T. chitinolytica*, while the lipolytic activity (lecithinase and lipase) of *T. chitinolytica* is more extensive than *T. mixta*. The carbon source utilization pattern of *T. mixta* (57 of 118 compounds utilized) is more extensive that *T. chitinolytica* (36 of 118 compounds utilized). Aromatic compounds are not utilized by *T. chitinolytica* (Bowman et al., 1993b).

Although no further isolations of *Telluria* have been published, a partial 16S rRNA gene sequence of *T. mixta* from a bacterial endophyte in potato was submitted to GenBank (GenBank accession number AF297697) by van Elsas, van Overbeek and Garbeva (MIBU, PRI, Binenhaven 5, Wageningen 6700, The Netherlands) in 2000 supporting the association of the species with the plant rhizosphere.

ENRICHMENT AND ISOLATION PROCEDURES

Cultures of *T. mixta* are best enriched from soil or rhizosphere in a medium containing dextran minerals salts (Blackall et al., 1985; Bowman et al., 1988). Isolation of pure cultures is readily achieved by plating from the enrichment on dextran agar and observing for colonies surrounded by a clear zone after flooding with ethanol indicative of dextran hydrolysis (Bowman et al., 1988). Colonies are circular, low convex, with a rough or smooth surface, white to tan in color, and may have a highly elastic and cartilaginous consistency. *T. chitinolytica* was first isolated from potted soil emended with powdered crustacean shells kept in a glasshouse at 27–29°C for up to 45 d. After enrichment, isolation was made by plating on agar plates containing 0.2% (w/v) colloidal chitin as the sole carbon source and selecting yellow-pigmented colonies producing a halo of chitin degradation (Spiegel et al., 1991). Colonies of *Telluria* on isolation are highly elastic and cartilaginous.

MAINTENANCE PROCEDURES

Media containing carbohydrates are required for good growth and maintenance. Sucrose peptone agar (Hayward, 1964) is a good general medium for maintenance, and good growth occurs on glucose nitrate medium. Dextran agar and chitin agar are also suitable for *T. mixta* and *T. chitinolytica*, respectively, and for checking culture purity (Bowman et al., 1988, 1993b). Cultures may be preserved by cryogenic storage in liquid nitrogen when suspended in sucrose peptone broth containing 10% glycerol, and by freeze drying in glucose peptone broth containing horse serum.

DIFFERENTIATION OF THE GENUS *TELLURIA* FROM OTHER GENERA

The primary characters for differentiation of *Telluria* from other high mol% G + C, strictly oxidative aerobes in the *Betaproteobacteria* are possession of mixed flagellation on solid agar and the inability to grow well on nutrient agar without the addition of carbohydrates. Additional characters useful for differentiation from *Ralstonia*, *Burkholderia*, *Comamonas*, *Delftia*, *Acidovorax*, *Hydrogenophaga*, *Variovorax*, and *Xylophilus* are given in Table BXII.β.28.

TAXONOMIC COMMENTS

Phylogenetically, the genus *Telluria* belongs to a distinct, well-supported branch in the *Betaproteobacteria* comprising the two species of *Telluria* with 97% rDNA sequence similarity, and the species *Duganella zoogloeoides*, *Janthinobacterium lividum*, *Herbaspirillum* species, and *Paucimonas lemoignei* (Fig. BXII.β.20). The nearest relatives of *Telluria* species are *Duganella zoogloeoides* and *Janthinobacterium lividum*, which are only moderately related at 93% sequence similarity to *Telluria*. These phylogenetic relationships confirm studies by Bowman et al. (1993b), Anzai et al. (2000), and Jendrossek (2001).

DIFFERENTIATION OF THE SPECIES OF THE GENUS *TELLURIA*

General characteristics of the species are given in the generic description. Differential characteristics of the species of *Telluria* are indicated in Table BXII.β.27, and additional characteristics are provided in the section on further descriptive information.

List of species of the genus Telluria

1. **Telluria mixta** (Bowman, Sly and Hayward 1989) Bowman, Sly, Hayward, Spiegel and Stackebrandt 1993b, 124[VP] (*Pseudomonas mixta* Bowman, Sly and Hayward 1989, 205.)
   mix′ta. L. adj. *mixtus* mixed; M.L. fem. adj. *mixta* mixed, referring to mixed flagellation.

   Colonies are circular, low convex, with a rough or smooth surface, white to tan in color, and may have a highly elastic and cartilaginous consistency. Unable to grow at 45°C. Nitrate is reduced to nitrite. Urease and β-galactosidase positive. Lecithinase not produced. Dextran, pectate, and Tween 20 are hydrolyzed, but not chitin or tributyrin. DL-Arabinose, lactose, dextran, butyrate, DL-glycerate, glycolate, benzoate, *p*-hydroxybenzoate, DL-serine, DL-threonine, L-arginine, L-citrulline, L-ornithine, and L-phenylalanine are utilized as carbon sources, but not glycerol or acetamide. L-Tyrosine, poly-β-hydroxybutyrate, heptanoate,

caprylate, mannitol, phenol, *m*-hydroxybenzoate, benzoylformate, DL-mandelate, and quinate are utilized by most strains. Isolated from various soils in Queensland, Australia.

   The mol% G + C of the DNA is: 69 (T_i).
   Type strain: ACM 1762, ATCC 49108, UQM 1762.
   GenBank accession number (16S rRNA): X65589.

2. **Telluria chitinolytica** Bowman, Sly, Hayward, Spiegel and Stackebrandt 1993b, 124[VP]
   chi.tin.o.lyt′i.ca. *chitin* clinical term for a polysaccharide; Gr. adj. *lytos* soluble; M.L. adj. *lytos* soluble; M.L. fem. adj. *chitinolytica* dissolving chitin.

   Colonies are yellow-pigmented and on isolation are highly elastic and cartilaginous. Able to grow at 45°C. Nitrate is not reduced to nitrite. Urease and β-galactosidase negative. Lecithinase is produced. Chitin and tributyrin are

TABLE BXII.β.28. Differentiation of the genus *Telluria* from other high mol% G + C, strictly oxidative aerobes in the *Betaproteobacteria*[a,b]

Characteristic	Telluria	Ralstonia	Burkholderia	Comamonas	Delftia	Acidovorax	Hydrogenophaga	Variovorax	Xylophilus
Flagellation:									
Mixed (1 polar and >1 lateral)	+	−	−	−	−	−	−	−	−
Polar (≥1)	+	D	D	+	+	+	+	−	+
Peritrichous	−	D	−	−	−	−	−	+	−
Bipolar tufts	−	−	−	+	+	−	−	−	−
Pigments	D	D	D	−	−	−	+	+	+
Hydrogen autotrophy	−	D	−	−	−	D	+	d	−
Oxidase	+	D	D	+	+	+	+	+	−
Hydrolysis of starch	+	D	D	−	−	−	−	−	d
Growth on nutrient agar	−	+	+	+	+	+	+	+	−
Tolerance to 1.5% NaCl	−	+		+	+	+	+	+	−
Utilization of:									
Glucose	+	D	+	−	−	D	+	+	+
Fructose	+	D	+	−	+	+	D		−
Occurrence[c]	S	S, FW, CS, IP	S, FW, CS, IP	S, FW, CS	S, FW, CS	S, FW, CS	S, FW	S, FW	IP
Mol% G + C	67–72	64–69	59–70	63–66	67–69	62–66	65–69	66–68	68–69

[a]Symbols: +, present in all species; −, absent in all species; (+), weak reaction; d,11–89% of strains are positive; D, variable reaction in different species.

[b]Data from Bradbury (1984), Kersters and De Ley (1984b), Palleroni (1984), De Vos et al. (1985b), Tamaoka et al. (1987), Willems et al. (1987, 1989, 1990, 1991a, c, 1992a), Bowman et al. (1988, 1993b), Gillis et al. (1995), Urakami et al. (1995a), Yabuuchi et al. (1995), Zhang et al. (2000a), Brämer et al. (2001), Chen et al. (2001), Coenye et al. (2001a, c), and Goris et al. (2001).

[c]S, soil; FW, fresh water; CS, clinical sample; IP, infected plants.
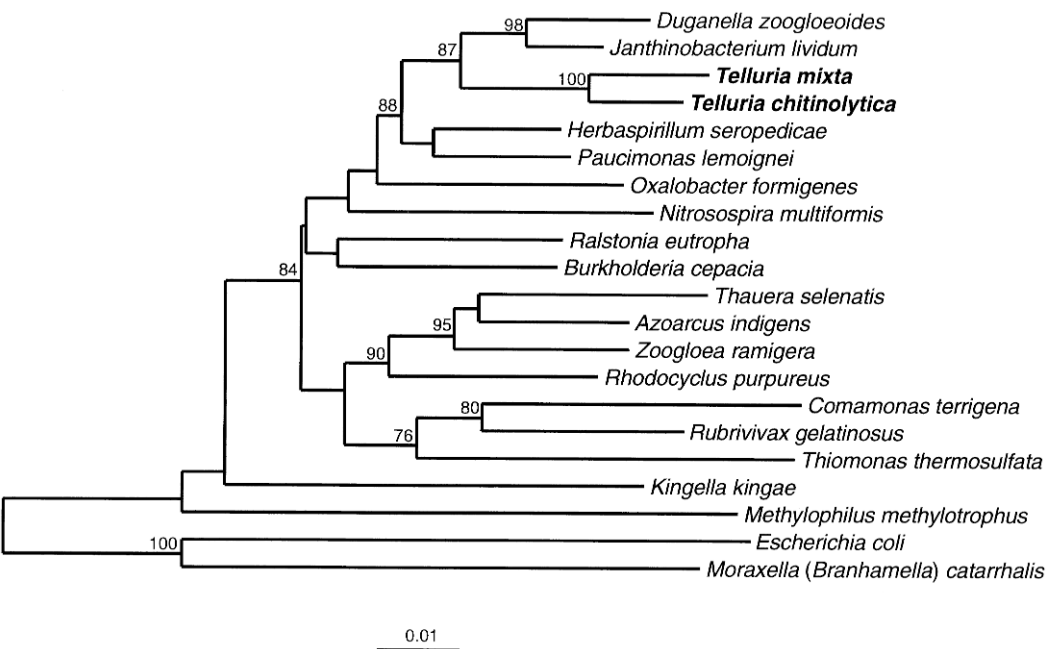
FIGURE BXII.β.20. Neighbor-joining tree reconstructed from 16S rRNA gene sequences using the PHYLIP programs (Felsenstein, 1993) as implemented in the ARB program (Software available from O. Strunk and W. Ludwig: ARB: a software environment for sequence data). Bootstrap values of 100 resamplings are shown at the branch points. Scale bar represents 1 nucleotide substitution per 100 nucleotide positions. The tree was constructed using the following sequences: *Duganella zoogloeoides* IAM 12670[T], D14256; *Janthinobacterium lividum* DSM 1522[T], Y08846; *Telluria mixta* ACM 1762[T], X65589; *Telluria chitinolytica* ACM 3522[T], X65590; *Herbaspirillum seropedicae* ATCC 35892[T] (DSM 6445[T]), Y10146; *Paucimonas lemoignei* ATCC 17989[T], AB021375; *Oxalobacter formigenes* OxB[T], U49757; *Nitrosospira multiformis* ATCC 25196[T], L35509; *Ralstonia eutropha* ATCC 17697[T], 335 (Stanier), M32021; *Burkholderia cepacia* ATCC 25416[T], M22518; *Thauera selenatis* ATCC 55363[T], X68491; *Azoarcus indigens* VB 32[T], L15531; *Zoogloea ramigera* ATCC 19544[T] (NCIMB 10706[T]) X74913; *Rhodocyclus purpureus* DSM 168[T], M34132; *Comamonas terrigena* IMI 359870[T], AF078772; *Rubrivivax gelatinosus* ATCC 17011[T] (DSM 1709[T]) D16213; *Thiomonas thermosulfata* ATCC 51520[T], U27839; *Kingella kingae* ATCC 23330[T], M22467; *Methylophilus methylotrophus* AS1[T], M29021; *Escherichia coli* ATCC 11775[T], X80725; *Moraxella catarrhalis* ATCC 25238[T], U10876.

hydrolyzed, but not dextran, pectate, or Tween 20. Glycerol and acetamide are utilized as carbon sources, but not DL-arabinose, lactose, dextran, butyrate, DL-glycerate, glycolate, benzoate, *p*-hydroxybenzoate, DL-serine, DL-threonine, L-arginine, L-citrulline, L-ornithine, and L-phenylalanine. L-tyrosine, poly-β-hydroxybutyrate, heptanoate, caprylate, man-

nitol, phenol, *m*-hydroxybenzoate, benzoyl-formate, DL-mandelate, and quinate are not utilized. The type strain was isolated from a loamy soil from Bet Dagan, Israel.

The mol% G + C of the DNA is: 72 (T_i).
Type strain: ACM 3522, CNCM I-804.
GenBank accession number (16S rRNA): X65590.

Family III. **Alcaligenaceae** De Ley, Segers, Kersters, Mannheim and Lievens 1986, 412[VP]

HANS-JÜRGEN BUSSE AND GEORG AULING

Al.cal.li.ge.na' ce.ae. M.L. masc. n. *Alcaligenes* type genus of the family; M.L. masc. pl. n. *Alcaligenaceae* the *Alcaligenes* family.

Rods or coccobacilli 0.2–1.0 × 0.5–2.6 μm, occurring singly, in pairs, or rarely in chains. **Gram negative**. No resting stages are known. Motile by peritrichous flagella or nonmotile (*Bordetella parapertussis* and *Bordetella pertussis*). Aerobic, having a **strictly respiratory** type of metabolism with oxygen as the terminal electron acceptor. Some strains can use nitrate or nitrite as an alternative electron acceptor, allowing growth to occur anaerobically. Optimal temperature, 30–37°C. Colonies are usually nonpigmented. Most species are oxidase and catalase positive. Alkaline reaction in litmus milk. Gelatin not hydrolyzed. **Chemoorganotrophic**. Most species utilize a variety of organic acids and amino acids as carbon sources. Carbohydrates are usually not utilized. *Achromobacter xylosoxidans* subsp. *xylosoxidans* produces acid from D-glucose and D-xylose and utilizes both of these carbohydrates as carbon sources. Some species require nicotinamide, organic sulfur (e.g., cysteine), and organic nitrogen (amino acids).

As demonstrated by DNA–rRNA hybridization experiments (De Ley et al., 1986) and 16S rDNA gene sequence analyses (Vandamme et al., 1996a), the family *Alcaligenaceae* belongs to the *Betaproteobacteria*. In general, species of the family *Alcaligenaceae* are characterized by **ubiquinone-8** (Q-8) as the major isoprenoid quinone (Fletcher et al., 1987; Oyaizu-Masuchi and Komagata, 1988). Species analyzed for their polyamine content contain **putrescine** as the major compound and the β-subclass-specific polyamine **2-hydroxyputrescine** (HPUT) (Busse and Auling, 1988; Hamana and Takeuchi, 1998). All species contain in their fatty acid profile the major compounds **hexadecanoate** ($C_{16:0}$) and **cycloheptadecanoate** ($C_{17:0 \text{ cyclo}}$), but *Bordetella pertussis* lacks the acid $C_{17:0 \text{ cyclo}}$ (Oyaizu-Masuchi and Komagata, 1988; Vancanneyt et al., 1995; Weyant et al., 1995a; Vandamme et al., 1996a).

Species of the genera *Alcaligenes* and *Achromobacter* occur in soil, water, and the hospital environment and have been isolated from human clinical material. Species of the genus *Bordetella* have been isolated from humans and warm-blooded animals but not all are pathogenic for warm-blooded animals (*Bordetella trematum*). The single species of the genus *Pigmentiphaga* has been isolated from soil.

The mol% G + C of the DNA is: 56–70.

Type genus: **Alcaligenes** Castellani and Chalmers 1919, 936.

FURTHER DESCRIPTIVE INFORMATION

The family *Alcaligenaceae* was proposed by De Ley et al. (1986) to encompass the genera *Alcaligenes* and *Bordetella*, which were shown by DNA–rRNA hybridization to group in one cluster within the *Betaproteobacteria*. Phylogenetic analyses based on 16S rDNA sequence data confirm this clustering (Weyant et al., 1995a). Recently, the species *Alcaligenes denitrificans*, *Alcaligenes xylosoxidans*, and *Alcaligenes piechaudii* have been reclassified in the genus *Achromobacter* (Yabuuchi et al., 1998a). Sequence alignment to 120 primary structures of 23S rDNAs from other members of the domain *Bacteria* representing all known phyla confirmed a closer relationship between *Bordetella* species and the type species of the genus, *Alcaligenes faecalis*, in a stable subtree comprising the *Betaproteobacteria* (Ludwig et al., 1995). In this context it is of interest that species of both *Achromobacter* and *Bordetella* produce the macrocyclic siderophore alcaligin, which was originally isolated from *Alcaligenes xylosoxidans* (Nishio et al., 1988; Nishio and Ishida, 1990), and the alcaligin biosynthesis genes have been characterized in *Bordetella pertussis* (Kang et al., 1996).

On the other hand, 16S rDNA sequence data also demonstrate the distance of certain species to the family *Alcaligenaceae*, previously transferred from the genus *Alcaligenes* to other genera outside the family (Auling et al., 1988; Willems et al., 1991a; Dobson et al., 1993; Meyer et al., 1993; Yabuuchi et al., 1995; Dobson and Franzmann, 1996), such as *Ralstonia eutropha* (basonym *Alcaligenes eutrophus*), *Variovorax paradoxus* (basonym *Alcaligenes paradoxus*), *Halomonas aquamarina* (basonym *Alcaligenes aquamarinus*; basonym *Deleya aquamarina*), *Halomonas cupida* (basonym *Alcaligenes cupidus*; basonym *Deleya cupida*), *Halomonas pacifica* (basonym *Alcaligenes pacificus*; basonym *Deleya pacifica*), *Halomonas venusta* (basonym *Alcaligenes venustus*; basonym *Deleya venusta*), and *Carbophilus carboxidus* (formerly "*Alcaligenes carboxidus*"). The species reclassified as members of the genera *Ralstonia*, *Halomonas*, and *Carbophilus* can be easily distinguished from the genus *Alcaligenes* based on their fatty acid profiles, quinones, and/or polyamine patterns (Table BXII.β.29). *V. paradoxus* might be distinguished from members of the family *Alcaligenaceae* by its yellow pigmentation, utilization of the carbon sources D-galactose, L-arabinose, D-mannitol, and *p*-hydroxybenzoate, and the presence of urease (Kersters and De Ley, 1984b). Results from DNA–rRNA hybridization studies have demonstrated that *Alcaligenes latus* is most likely misnamed and a member of an as yet undescribed genus (Willems et al., 1991b). Differentiation of *A. latus* from the species of the genus *Alcaligenes* may be achieved by its ability to utilize maltose, sucrose, 2-ketogluconate, *p*-hydroxybenzoate, butylamine, betaine, sarcosine, and creatine as a carbon source, by chemolithotrophic growth with molecular hydrogen, and by hydrolysis of gelatin, starch, and Tween 80 (Kersters and De Ley, 1984b).

The genus *Bordetella* consists of seven species: *B. pertussis* (Moreno-López, 1952), *B. parapertussis* (Eldering and Kendrick, 1938), *B. bronchiseptica* (Moreno-López, 1952), *B. avium* (Kersters et al., 1984), *B. hinzii* (Vandamme et al., 1995c), *B. holmesii* (Weyant et al., 1995a), and *B. trematum* (Vandamme et al., 1996a). DNA–DNA hybridization (Kloos et al., 1981), multilocus enzyme electrophoresis (Musser et al., 1986), ERIC-PCR, and ARDRA (Vandamme et al., 1997a) studies have shown that the species *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* are closely related and may be considered different subspecies of a single species. In contrast, macrorestriction digests resolved by pulsed-field gel electrophoresis (Khattak and Matthews, 1993), whole-cell protein electrophoresis, and fatty acid profiles (Vancanneyt et al., 1995) generated species-specific profiles for these three species, supporting their present status as different species. Interestingly, it has been independently shown by two groups (Vancanneyt et al., 1995; Weyant et al., 1995a) that *B. pertussis* lacks the fatty acid $C_{17:0\ cyclo}$, which is a predominant compound in the fatty acid profile of the majority of members of the family. Thus, the important human pathogen *B. pertussis* can be easily identified by fatty acid analysis. The taxonomic status of the remaining species *B. avium*, *B. hinzii*, *B. holmesii*, and *B. trematum* is not a matter of discussion. These four species are phenotypically and genotypically sufficiently distinct from each other and from the other three species to justify their status as single species (Vandamme et al., 1997a).

Until the recent reclassification of the species *Alcaligenes denitrificans*, *Alcaligenes xylosoxidans*, and *Alcaligenes piechaudii* to the genus *Achromobacter* as *Achromobacter xylosoxidans* subsp. *denitrificans*, *Achromobacter xylosoxidans* subsp. *xylosoxidans*, and *Achromobacter piechaudii* (Yabuuchi et al., 1998a), taxonomic confusion arose from the phylogenetic clustering of species within the family. Based on 16S rDNA sequence comparisons, the species *Achromobacter xylosoxidans* subsp. *denitrificans*, *Achromobacter xylosoxidans* subsp. *xylosoxidans*, and *Achromobacter piechaudii* are more closely related to the type species of the genus *Bordetella* than to the type species of the genus *Alcaligenes*, *Alcaligenes faecalis* (Fig. BXII.β.21). Likewise, the newly described species *Alcaligenes defragrans* (Foss et al., 1998a) is phylogenetically clearly separated from all genera within the family *Alcaligenaceae* (Blümel et al., 2001b). Thus, it is most likely that *A. defragrans* will be reclassified as a member of a new genus within the family. The DNA base ratios reflect the separate position of *A. faecalis*. While *A. xylosoxidans* subsp. *denitrificans*, *A. xylosoxidans* subsp. *xylosoxidans*, *A. ruhlandii*, *A. piechaudii*, and *A. defragrans* have a mol% G + C content (64–70%) in the range of *Bordetella* species (62–70%), a lower DNA base ratio of 56–60% has been reported for *A. faecalis* (De Ley et al., 1986). Thus, the generic homogeneity within the family has been enhanced by removal of *A. denitrificans*, *A. xylosoxidans*, and *A. piechaudii* from the genus *Alcaligenes*. From the phylogenetic point of view, it would be acceptable to transfer the species *A. xylosoxidans* subsp. *denitrificans*, *A. xylosoxidans* subsp. *xylosoxidans*, *A. piechaudii*, and *A. defragrans* into the genus *Bordetella*. One major obstacle to this reclassification is the definition of the genus *Bordetella*, which describes its members as mammalian parasites and pathogens living among epithelial cilia of the respiratory tract (Pittman, 1984b). However, no association with the respiratory tract of any of the *Alcaligenes* and *Achromobacter* species in question has been described so far. Only *Achromobacter xylosoxidans* subsp. *xylosoxidans* is an important opportunistic pathogen as concluded from numerous reports (Reverdy et al., 1984; Chandrasekar et al., 1986; Reina et al., 1988;

Schoch and Cunha, 1988; Legrand and Anaissie, 1992; Cheron et al., 1994; Dunne and Maisch, 1995; Knippschild et al., 1996). The species *A. ruhlandii* and *A. defragrans* have not been isolated from clinical specimens (Foss et al., 1998a). In the last decade, new *Bordetella* species were isolated from the respiratory tract of birds, human blood cultures, ear infections, and wounds of humans (*B. avium* [Kersters et al., 1984], *B. hinzii* [Vandamme et al., 1995c], *B. holmesii* [Weyant et al., 1995a], and *B. trematum* [Vandamme et al., 1996a]). However, no pathogenic significance was demonstrated for *B. trematum*, and that of *B. holmesii* might be limited to opportunistic infections. *B. avium* is a pathogen only for animals. An eventual emendation of *Bordetella* to a more broadly defined genus that is not restricted to obligate pathogens that thrive between epithelial cilia of the respiratory tract would be an alternative to solve discrepancies between phylogeny and taxonomy. This would allow inclusion of the species *A. xylosoxidans* subsp. *denitrificans*, *A. xylosoxidans* subsp. *xylosoxidans*, and *A. piechaudii*, often isolated from clinical specimens, and even *A. ruhlandii*, into a redefined genus *Bordetella*.

Very recently, the genus *Pigmentiphaga* with the single species *Pigmentiphaga kullae* has been proposed as a new genus of the family *Alcaligenaceae* (Blümel et al., 2001b). The single strain of *P. kullae* was isolated after an aerobic enrichment with the azo compound 1-(4′-carboxyphenylazo)-4-naphthol as sole source of carbon and energy (Kulla et al., 1984). Analysis of the 16S rDNA sequence (95–96% similarity with members of the family *Alcaligenaceae*), designation of signature nucleotides, and chemotaxonomic characteristics (presence of ubiquinone Q-8, the polyamines putrescine and 2-hydroxyputrescine, and the fatty acids $C_{16:0}$ and $C_{17:0\ cyclo}$) placed *P. kullae* unambiguously within the family *Alcaligenaceae*.

Some taxonomic comments have to be addressed regarding *Taylorella equigenitalis*, *Taylorella asinigenitalis*, and *Pelistega europaea*. *T. equigenitalis* was originally described as *Haemophilus equigenitalis* (Taylor et al., 1978). A reinvestigation of the taxonomic position of *H. equigenitalis* based on phenotypic data, DNA base composition, and DNA–DNA hybridization data led to its transfer to the new genus *Taylorella*, as its type species *T. equigenitalis* (Sugimoto et al., 1983). The second species of this genus, *T. asinigenitalis*, shares 97.6% 16S rDNA sequence similarity with strains of *Taylorella equigenitalis* (Jang et al., 2001). 16S rDNA sequence analyses (Bleumink-Pluym et al., 1993; Jang et al., 2001) demonstrated that the genera *Pelistega*, *Alcaligenes*, *Achromobacter*, and *Bordetella* are the closest phylogenetic relatives of the genus *Taylorella*. As deduced from phylogenetic analyses based on 16S rDNA sequences, *T. equigenitalis* and *A. faecalis* share approximately the same distance to certain *Bordetella* species and *A. xylosoxidans* subsp. *xylosoxidans* (Bleumink-Pluym et al., 1993; Weyant et al., 1995a). These data indicate that the genus *Taylorella* may represent another genus within the family *Alcaligenaceae*. However, its inclusion in the family is not clearly supported by other data. *Taylorella* species have been shown to have a DNA base composition of ~38 mol% G + C. This value is substantially lower than the mol% G + C content of *A. faecalis*, which is known to have the lowest mol% G + C content (57.4% as determined for the type strain) within the family. Likewise, the fatty acid profile of *Taylorella* species with the major compounds $C_{18:1}$, $C_{16:0}$, $C_{18:0}$, and $C_{14:0\ 3OH}$ (Rossau et al., 1987; Jang et al., 2001) does not reflect the characteristics of the family, i.e., the compound $C_{17:0\ cyclo}$ (Table BXII.β.29) is missing and the predominant compound, $C_{18:1}$, is not a major fatty acid in the profile of the family members. However, the presence of $C_{16:0}$ and $C_{14:0\ 3OH}$ fatty acids

TABLE BXII.β.29. Differentiating characteristics of *Alcaligenes* species and species that previously have been transferred from the genus *Alcaligenes* to other genera, including *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*), *Variovorax paradoxus* (formerly *Alcaligenes paradoxus*), *Halomonas aesta* (formerly *Alcaligenes aestus*, *Deleya aesta*), *Halomonas aquamarina* (formerly *Alcaligenes aquamarinus*, *Deleya aquamarina*), *Halomonas cupida* (formerly *Alcaligenes cupidus*, *Deleya cupida*), *Halomonas pacifica* (formerly *Alcaligenes pacificus*, *Deleya pacificus*), *Halomonas venusta* (formerly *Alcaligenes venustus*, *Deleya venusta*), and *Carbophilus carboxidus* (formerly "*Alcaligenes carboxidus*")

Characteristic	*Alcaligenes faecalis*[a]	*Alcaligenes defragrans*[b]	*Alcaligenes latus*[c]	*Alcaligenes xylosoxidans* subsp. *denitrificans*[a]	*Alcaligenes xylosoxidans* subsp. *xylosoxidans*[d]	*Achromobacter ruhlandii*[e]	*Achromobacter piechaudii*[f]	*Ralstonia eutropha*[g]	*Variovorax paradoxus*[h]	*Halomonas aquamarina*[h]	*Halomonas cupida*[i]	*Halomonas pacifica*[i]	*Halomonas venusta*[i]	*Carbophilus carboxidus*[j]
Major fatty acids	$C_{17:0}$ cyclo, $C_{16:0}$	$C, C_{16:1}$, $C_{16:0}$, $C_{18:1}$,[k] $C_{17:0}$ cyclo	$C_{16:1}$, $C_{18:1}$, $C_{16:0}$	$C_{16:0}$, $C_{17:0}$ cyclo	$C_{16:0}$, $C_{17:0}$ cyclo		$C_{17:0}$ cyclo, $C_{16:0}$	$C_{16:1}$, $C_{16:0}$	$C_{16:0}$, $C_{17:0}$ cyclo ($C_{15:0}$, $C_{16:1}$, $C_{16:0}$, $C_{18:1}$)	$C_{18:1}$, $C_{16:0}$, $C_{19:0}$ cyclo	$C_{18:1}$, $C_{16:0}$, $C_{19:0}$ cyclo	$C_{18:1}$, $C_{16:0}$, $C_{19:0}$ cyclo	$C_{18:1}$, $C_{16:0}$	$C_{18:1}$, $C_{16:0}$
Hydroxy fatty acids	$C_{14:0}$ 3OH, $C_{12:0}$ 2OH	$C_{14:0}$ 3OH	$C_{10:0}$ 3OH	$C_{14:0}$ 3OH, $C_{12:0}$ 2OH, $C_{16:0}$ 2OH	$C_{14:0}$ 3OH, $C_{12:0}$ 2OH, $C_{16:0}$ 2OH		$C_{14:0}$ 3OH, $C_{12:0}$ 2OH, $C_{16:0}$ 2OH	$C_{14:0}$ 3OH, $C_{14:0}$ 2OH, $C_{12:0}$ 3OH	$C_{12:0}$ 3OH, $C_{10:0}$ 3OH, $C_{16:0}$ 3OH ($C_{10:0}$ 3OH, $C_{14:0}$ 3OH, $C_{8:0}$ 3OH)[m]	$C_{12:0}$ 3OH	$C_{12:0}$ 3OH	$C_{12:0}$ 3OH	$C_{12:0}$ 3OH	$C_{12:0}$ 3OH
Respiratory quinone	Q-8		Q-8	Q-8	Q-8			Q-8	Q-8	Q-9	Q-9	Q-9	Q-9	Q-10
Characteristic polyamines[n]	PUT, HPUT			PUT, HPUT	PUT, HPUT	PUT, HPUT		PUT, HPUT	PUT, HPUT	SPD	SPD	SPD	SPD	HSPD, PUT
Yellow pigment	–	–	–	–	–		–	–	+	–	–	–	–	–
Chemolithotrophic growth on H_2	–	+	+	–	–	+	–	+	d	Not analyzed	–	–	–	–
Urease	–			–	–	–	–	–	+	–	–	–	–	–
Hydrolysis of:														
Gelatin	–		+	–	–	–	–	–	d	–	–	–	–	–
Starch	–		+	–	–	–	–	–	–	d	–	–	–	–
Tween 80	–		+	–	–	–	–	+	+					–

(continued)

TABLE BXII.β.29. *(cont.)*

Characteristic	*Alcaligenes faecalis* [a]	*Alcaligenes defragrans* [b]	*Alcaligenes latus* [c]	*Alcaligenes xylosoxidans* subsp. *denitrificans* [d]	*Alcaligenes xylosoxidans* subsp. *xylosoxidans* [d]	*Achromobacter ruhlandii* [e]	*Achromobacter piechaudii* [f]	*Ralstonia eutropha* [g]	*Variovorax paradoxus* [h]	*Halomonas aquamarina* [h]	*Halomonas cupida* [i]	*Halomonas pacifica* [i]	*Halomonas venusta* [i]	*Carbophilus carboxidus* [j]
Carbon source for growth:														
D-Galactose	−		−	−	+	+	Not analyzed	−	+	d	+	−	−	+
L-Arabinose	−		−	−	−	−	−	−	+	−	+	−	−	
D-Mannitol	−		−	−	−	−	−	−	+	+	+	−	[+]	+
Maltose	−		+	−	−	−	−	−	−	d	d	−	d	+
Sucrose	−	−	+	−	−		Not analyzed	−	−	d	d	−	d	+
2-Keto-gluconate	−		+	−	−	−	Not analyzed	+	d	−				
p-Hydroxy-benzoate	−		+	−	−	+		+	+	−	+	d	d	
Butylamine	−		+	−	−			−	d	−	−	+	d	
Betaine	−		+	−	−			−	−	−	+	−	+	+
Sarcosine	−		+	−	−			−	−	−	−	+	+	
Creatine	−		+	−	−			−			−	−	d	
Mol% G + C of DNA	55.9–59.4	66.9	69.1–71.1	63.9–68.9	66.0–69.8	67.7	64.0–65.0	66.3–67.5	66.8–69.4	56.9–57.9	59.9–62.1	67.3–68.3	52.9–54.5	62.8

[a] Data from Gilardi (1978a); Pichinoty et al. (1978); Rarick et al. (1978); Rubin et al. (1980); Kiredjian et al. (1981); Yamasato et al. (1982); Kersters and De Ley (1984b); Busse and Auling (1988); Lipski et al. (1992); Vandamme et al. (1995c); Hamana and Takeuchi (1998).

[b] Data from Foss et al. (1998a).

[c] Data from Palleroni and Palleroni (1978); Oyaizu-Masuchi and Komagata (1988).

[d] Data from Yabuuchi et al. (1974); Gilardi (1978a); Pichinoty et al. (1978); Rarick et al. (1978); Rubin et al. (1980); Kiredjian et al. (1981) Yamasato et al. (1982); Kersters and De Ley (1984b); Busse and Auling (1988); Oyaizu-Masuchi and Komagata (1988); Vandamme et al. (1995c); Hamana and Takeuchi (1998).

[e] Data from Yamasato et al. (1982); Busse and Auling (1988); Yabuuchi et al. (1998a); Hamana and Takeuchi (1998).

[f] Data from Kiredjian et al. (1986); Vandamme et al. (1995c); Hamana and Takeuchi (1998).

[g] Data from Davis et al. (1969, 1970); Kersters and De Ley (1984b); Busse and Auling (1988); Oyaizu-Masuchi and Komagata (1988); Lipski et al. (1992).

[h] Data from ZoBell and Upham (1944); Baumann et al. (1972); Holding and Shewan (1974); Kersters and De Ley (1984b); Franzmann and Tindall (1990); Auling et al. (1991); Akagawa-Matsushita et al. (1992); Hamana (1997). The data include the characteristics of *D. aesta* recently reclassified as *H. aquamarina* (Akagawa and Yamasato, 1989).

[i] Data from Baumann et al. (1972); Franzmann and Tindall (1990); Akagawa-Matsushita et al. (1992); Hamana (1997).

[j] Data from Auling et al. (1988); Meyer et al. (1993).

[k] The fatty acid composition was analyzed from biomass that was grown under denitrifying conditions with acetate as the sole carbon source.

[l] In the original paper (Vandamme et al., 1995c) the authors pointed out that the microbial identification system (Microbial ID, Inc., Newark, Delaware) used for identification of fatty acids cannot distinguish between the two acids $C_{16:1\ iso}$ and $C_{14:0\ 3OH}$. Since branched-chain fatty acids have not been reported in *Alcaligenes* or *Bordetella* strains or are present only in trace amounts, the peak designated summed in feature 3 most probably corresponds to $C_{14:0\ 3OH}$.

[m] The values in brackets indicate the differing results as determined by Willems et al. (1989).

[n] PUT, putrescine; HPUT, 2-hydroxyputrescine; SPD, spermidine; HSPD, *sym*-homospermidine.
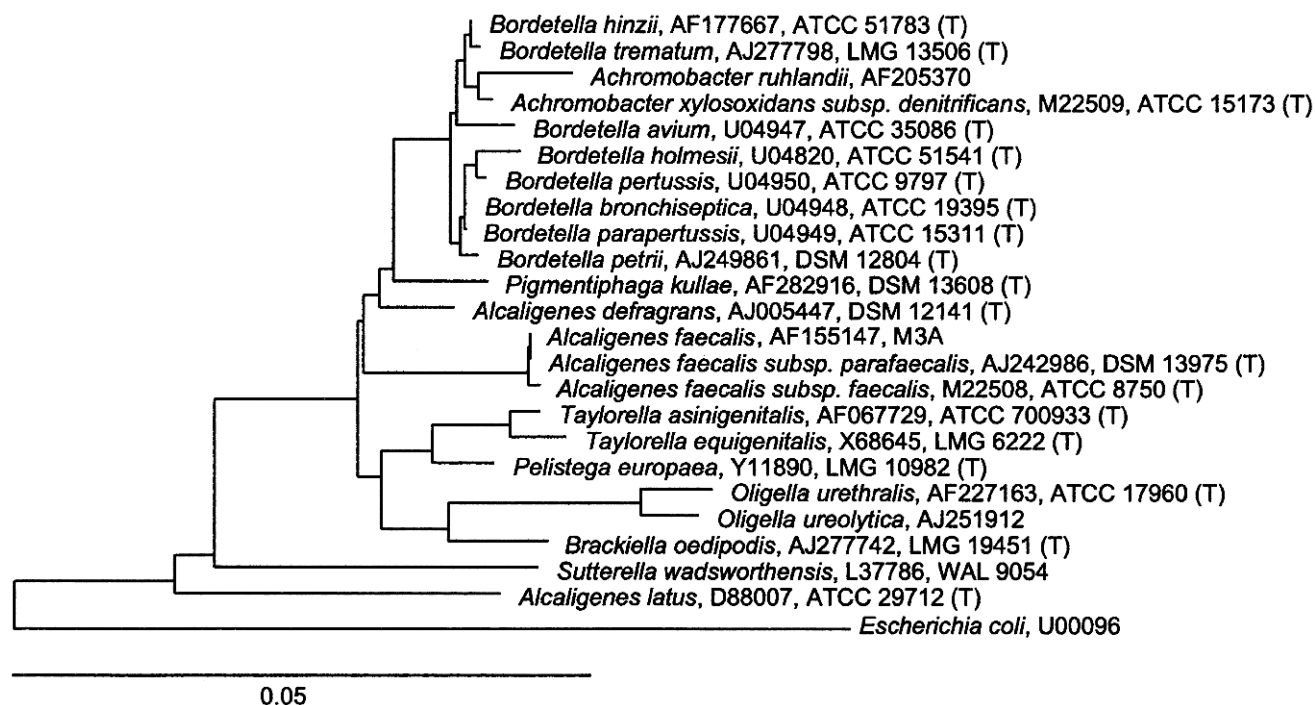
FIGURE BXII.β.21. Phylogenetic relationships of the family *Alcaligenaceae*. The distances in the tree were calculated using 1101 positions (the least-squares method, Jukes-Cantor model). (Courtesy T. Lilburn of the Ribosomal Database Project.)

as major compounds in *Taylorella* species is characteristic of all validly described species of the family *Alcaligenaceae*.

The closest phylogenetic relative of *Taylorella* is *Pelistega europaea* (Vandamme et al., 1998). Members of this species were reported to have a low mol% G + C content of genomic DNA (42–43%), and a fatty acid profile with the predominant acids $C_{18:1}$, $C_{16:1}$, $C_{16:0}$, summed feature 3 ($C_{14:0\ 3OH}$ or $C_{16:1\ iso\ I}$ or both), and $C_{14:0}$. *P. europaea* can be distinguished from members of the genus *Taylorella* based on 16S rDNA sequences dissimilarities (5.2%), and by qualitative and quantitative differences in the fatty acid profile and biochemical traits. To substantiate the allocation of *Taylorella* and *P. europaea* to an enlarged family *Alcaligenaceae*, the definition of signature nucleotides in the 16S rDNA sequence might be the most promising approach.

The genus *Sutterella* might be another member of the family *Alcaligenaceae* as deduced from 16S rDNA sequences of its single species *S. wadsworthensis* (Wexler et al., 1996a). This microaerophilic/anaerobic species has $C_{16:0}$ and $C_{16:1\ \omega7c}$ acids and summed feature 10 ($C_{18:1\ \omega7c,\ 9t,\ 12c}$ and/or an unknown peak). This profile and the relatively low mol% G + C content (36.5%) remind us of traits in members of *Taylorella* and *Pelistega*. Surprisingly, neither $C_{14:0\ 3OH}$ fatty acid present in all other members of the family *Alcaligenaceae* nor any other hydroxy fatty acid commonly associated to LPS of Gram-negative bacteria has been reported. On the other hand, inclusion of *Sutterella* into an emended family *Alcaligenaceae* appears questionable due to its phylogenetic equidistance (16S rDNA sequences similarities of 88–90%) to members of the family including *Taylorella* and *Pelistega* and to other members of the *Betaproteobacteria* such as *Ralstonia*, *Herbaspirillum*, and *Roseateles*.

Pathogenicity Strains of *A. faecalis*, *A. xylosoxidans* subsp. *denitrificans*, and *A. piechaudii* are frequently isolated from clinical specimens, and so they can be regarded as opportunistic path-

ogens (Kiredjian et al., 1981). Only *A. xylosoxidans* subsp. *xylosoxidans* is considered to be of clinical relevance. This species has been recognized as a causative agent of various human infections, mostly in immunocompromised hosts, i.e., patients with underlying diseases including endocarditis, meningitis, and ventriculitis after neurosurgery, pneumonia, bacteremia osteomyelitis, arthritis, and peritonitis (Shigeta et al., 1978; Reverdy et al., 1984; San Miguel et al., 1991; Legrand and Anaissie, 1992; Walsh et al., 1993). Several outbreaks of hospital-acquired infections have been observed in intensive care units or postsurgical recovery areas due to contamination with *A. xylosoxidans* subsp. *xylosoxidans* of antiseptic solutions (Shigeta et al., 1978), nonbacteriostatic solutions used in diagnostic tracer procedures (McGuckin et al., 1982), dialysis fluids (Reverdy et al., 1984), intravascular pressure transducers (Gahrn-Hansen et al., 1988), and a diagnostic contrast solution (Reina et al., 1988). Strains of *A. xylosoxidans* subsp. *xylosoxidans* have been reported to be susceptible to amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, azlocillin, ceftazidime, and imipenem, and resistant to cefazolin, cefuroxime, gentamicin, tobramycin, netilmicin, amikacin, tetracycline, ofloxacin, and ciproflxacin (Knippschild and Ansorg, 1998). The increasing recovery of this species from clinical specimens might be related to the extensive use of third-generation antibiotics such as cephalosporins and fluoroquinolones not effective against *A. xylosoxidans* subsp. *xylosoxidans* (Glupczynski et al., 1988; Mensah et al., 1989). No pathogenic potential has been reported for *A. ruhlandii*, *A. defragrans*, or *P. kullae*.

The genus *Bordetella* consists of several pathogenic bacteria. These bacteria produce many virulence related factors, among which there are several toxins such as the pertussis toxin (PTX) that is produced only by *B. pertussis*, the adenylate cyclase toxin (CYA), and several adhesins such as the filamentous hemagglutinin (FHA), the pertactin (PRN), and the fimbriae (Weiss, 1992). *B. pertussis* is the etiological agent of whooping cough

(Weiss and Hewlett, 1986). *B. parapertussis*, which causes a milder form of whooping cough, has been assumed as a strictly human pathogen (Hewlett, 1990), but it has been isolated from both healthy sheep and sheep affected with chronic nonprogressive pneumonia (Cullinane et al., 1987; Chen et al., 1988a; Porter et al., 1994). *B. bronchiseptica* is found in a wide range of animals, and it is associated with atrophic rhinitis in swine and kennel cough in dogs (Goodnow, 1980; Harkness and Wagner, 1995; Keil and Fenwick, 1998; Speakman et al., 1999). *B. avium* is a pathogen of birds and causes rhinotracheitis (turkey coryza) in turkey poults (Simmons and Gray, 1979; Simmons et al., 1979; Saif et al., 1980; Arp and Cheville, 1984; Kersters et al., 1984). Only recently, *B. hinzii* (Vandamme et al., 1995c) has been reported as the causative agent of fatal septicemia (Kattar et al., 2000). *B. holmesii* has been associated most often with septicemia in patients with underlying conditions (Lindquist et al., 1995; Weyant et al., 1995a; Morris and Meyers, 1998; Tang et al., 1998). It also has been isolated from sputum from one patient with respiratory symptoms. *B. trematum* has been isolated from wounds and ear infections, but its pathogenic significance is unknown (Vandamme et al., 1996a).

T. equigenitalis is considered a commensal or opportunistic pathogen. It colonizes the urogenital membranes of the clitoral sinuses and fossa, urethra, and cervix of mares, and the urethra, urethral fosses, and penile sheath of stallions. So far, only infections of mares have been reported where *T. equigenitalis* causes vaginal discharge, infertility, or early abortion (Wada et al., 1983). *T. equigenitalis* is moderately resistant to a limited number of antibiotics including clindamycin, lincomycin, trimethoprim, and sulfamethoxazole but sensitive to penicillin G, ampicillin, carbenicillin, cephaloridine, cephalothin, erythromycin, tetracycline, kanamycin, gentamicin, chloramphenicol, and polymyxin B (Sugimoto et al., 1983). *T. asinigenitalis* is not reported to cause disease in jacks or mares (Jang et al., 2001).

Pelistega europaea strains have been mainly isolated from lungs, air sac exudate, and trachea mucosa and less frequently from other organs such as liver and spleen of pigeons.

Sutterella wadsworthensis has been isolated from infections of gastrointestinal origin (Finegold and Jousimies-Somer, 1997; Jousimies-Somer, 1997). The majority of *Sutterella* strains are susceptible to amoxicillin/clavulanate, ticarcillin/clavulanate, cefoxitin, ceftriaxone, clindamycin, piperacillin/tazobactam, ceftizoxime, ciprofloxacin, trovafloxacin, azithromycin, clarithromycin, erythromycin, and roxithromycin.

Taxonomic Comments

The numerous reclassifications between the two genera *Alcaligenes* and *Achromobacter* suggest a common discussion of their history.

The type species of the genus *Alcaligenes* is *A. faecalis* Castellani and Chalmers 1919. This species also encompasses strains originally described as *"Pseudomonas odorans"* (Málek and Kazdová-Košisková, 1946) Málek et al., 1963, *"A. odorans* var. *viridans"* Mitchell and Clarke, 1965 and *"Achromobacter arsenoxydans-tres"* Turner, 1954.

Based on the phylogenetic relationships within the family *Alcaligenaceae*, *A. faecalis* can be considered as the only representative of the genus *Alcaligenes sensu stricto*. Recently, a strain accumulating poly-β-hydroxybutyrate from acetone-butanol bioprocess residues was shown to be closely related with *A. faecalis* DSM 30030^T (Schroll et al., 2001a). This strain shared 98.7% 16S rDNA sequence similarity, 56% DNA relatedness, and an almost identical protein pattern with the type strain of *A. faecalis*. Since it could only be distinguished from *A. faecalis* DSM 30030^T based on few physiological and biochemical traits and the extremely low content of the diagnostic polyamine 2-hydroxyputrescine, it was described as a subspecies of *A. faecalis*, *A. faecalis* subsp. *parafaecalis*. As a result of this study, the species *A. faecalis* is subdivided into the two subspecies *A. faecalis* subsp. *faecalis* and *A. faecalis* subsp. *parafaecalis*.

A. defragrans has been described as a new species (Foss et al., 1998a), encompassing four strains isolated on the alkenoic monoterpenes (+)-menthene, α-pinene, 2-carene, and α-phellandrene, respectively, as the sole source of carbon and energy under denitrifying conditions. 16S rDNA sequence data indicate that *A. defragrans* occupies a separate position within the *Alcaligenaceae*. Thus, future reclassification of this species in a new genus is most likely. In agreement with a proposal of a new genus for *A. defragrans* is its unusual fatty acid profile. In contrast to the fatty acid profiles of *Alcaligenes*, *Achromobacter*, and *Bordetella* species, *A. defragrans* was reported to contain significant amounts of dodecanal and to lack $C_{12:0\ 2OH}$ acid. However, the fatty acid profiles of strains of this species show the characteristics of the members of the family, the predominant acids $C_{16:0}$ and $C_{17:0\ cyclo}$, and the presence of the hydroxy acid $C_{14:0\ 3OH}$. Unfortunately, the fatty acid profile of *A. defragrans* was not obtained from cells grown on the commonly used trypticase soy agar. Thus, it cannot be compared directly with other profiles of species within the family *Alcaligenaceae* available from the literature and from the MIDI fatty acid identification system (Microbial ID, Newark, NJ, USA).

The type species of the genus *Achromobacter* is *A. xylosoxidans* (ex Yabuuchi and Ohyama 1971; Yabuuchi and Yano 1981) Yabuuchi et al. 1998a. The history of the two subspecies *Achromobacter xylosoxidans* subsp. *denitrificans* (formerly *Alcaligenes denitrificans* subsp. *denitrificans*) and *Achromobacter xylosoxidans* subsp. *xylosoxidans* (formerly *Alcaligenes denitrificans* subsp. *xylosoxidans*) has been reviewed in detail by Kersters and De Ley (1984b) and will be only summarized here. The name *Alcaligenes denitrificans* was proposed by Leifson and Hugh (1954a) to accommodate two strains that reduce nitrate to nitrite and gas. Based on biochemical and nutritional characteristics, Hendrie et al. (1974) proposed that *A. denitrificans* was a subjective synonym of *A. faecalis* Castellani and Chalmers 1919. The name was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980) and thus did not have standing in bacterial nomenclature. The name *A. denitrificans* was revived by Rüger and Tan (1983) to separate the original type strain of *A. denitrificans* (Leifson and Hugh, 1954a) from the species *A. faecalis*, based on DNA base composition, DNA reassociation, and nitrate reduction. In the first edition of *Bergey's Manual of Systematic Bacteriology*, *A. denitrificans* was named *A. denitrificans* subsp. *denitrificans*, *Achromobacter xylosoxidans* as *A. denitrificans* subsp. *xylosoxidans*, and *A. ruhlandii* was included in this second subspecies (Kersters and De Ley, 1984b). *Achromobacter xylosoxidans* was originally described by Yabuuchi and Ohyama (1971). Since this name was omitted from the Approved Lists of Bacterial names (Skerman et al., 1980), it had no standing in bacterial nomenclature. Thus, the name was revived by Yabuuchi and Yano (1981). Due to its high degree of similarity in the rRNA cistron to *A. denitrificans* subsp. *denitrificans*, Kersters and De Ley (1984b) proposed including as a second subspecies in *A. denitrificans*, namely *A. denitrificans* subsp. *xylosoxidans*. Additionally, the hydrogen-oxidizing species *A. ruhlandii* (Packer and Vishniac, 1955; Aragno and Schlegel,

1977), which appears in the Approved Lists, was transferred to *A. denitrificans* subsp. *xylosoxidans*, based on its similarity in genotypic and phenotypic features, and its indistinguishability from this subspecies in protein electrophoregrams.

Since the species epithet *xylosoxidans* had priority, Kiredjian et al. (1986) proposed the new combinations *A. xylosoxidans* subsp. *denitrificans* and *A. xylosoxidans* subsp. *xylosoxidans*. Vandamme et al. (1996a) demonstrated that strains of *A. xylosoxidans* subsp. *denitrificans* and *A. xylosoxidans* subsp. *xylosoxidans* can be readily distinguished by their whole-cell protein patterns, fatty acid components, and other phenotypic characteristics. These authors proposed that *A. xylosoxidans* subsp. *denitrificans* and *A. xylosoxidans* subsp. *xylosoxidans* should be elevated to species rank as *A. denitrificans* and *A. xylosoxidans*, respectively. Yabuuchi et al. (1998a) proposed combining *A. denitrificans* and *A. xylosoxidans* in a single species, *Achromobacter xylosoxidans*, with the two subspecies *Achromobacter xylosoxidans* subsp. *denitrificans* and *Achromobacter xylosoxidans* subsp. *xylosoxidans*, based on 63.4% DNA relatedness and 98.7% 16S rDNA sequence similarity between the type strains of the two subspecies. This proposal ignores the distinguishing characteristics reported by Vandamme et al. (1998) and thus creates a heterogeneous species.

The names *Alcaligenes denitrificans*, *Alcaligenes xylosoxidans* subsp. *denitrificans*, *Alcaligenes denitrificans* subsp. *denitrificans* and *Achromobacter xylosoxidans*, *Alcaligenes xylosoxidans*, *Alcaligenes xylosoxidans* subsp. *xylosoxidans*, and *Alcaligenes denitrificans* subsp. *xylosoxidans* are senior synonyms of the validated names *Achromobacter xylosoxidans* subsp. *denitrificans* and *Achromobacter xylosoxidans* subsp. *xylosoxidans*, respectively, as proposed by Yabuuchi et al. (1998a). Based on the low degree of DNA relatedness with other members of the genus *Achromobacter*, Yabuuchi et al. (1998a) also proposed the new species *Achromobacter ruhlandii* to accommodate the hydrogen-oxidizing strain of *Achromobacter xylosoxidans* originally described as "*Hydrogenomonas ruhlandii*" (Packer and Vishniac, 1955; Aragno and Schlegel, 1977).

The third species, *A. piechaudii*, encompasses isolates from human clinical material from different geographical regions (Kiredjian et al., 1986), as well as strains originally named *A. faecalis* CIB 60.75 and "*Achromobacter iophagus*" (Kiredjian et al., 1981; Holmes and Dawson, 1983), which were shown to be phenotypically and genotypically distinct from the other species transferred to the genus *Achromobacter*.

The marine *Alcaligenes* species *A. aestus*, *A. pacificus*, *A. cupidus*, and *A. venustus* have been reclassified and described as species of the new genus *Deleya* as *D. aesta*, *D. pacifica*, *D. cupida*, and *D. venusta* (Baumann et al., 1983). Akagawa and Yamasato (1989) showed that *A. aquamarinus* is another member of the genus *Deleya*. They also demonstrated that the type strains of *A. aquamarinus*, *A. faecalis* subsp. *homari* (Austin et al., 1981), and *D. aesta* are members of the same species, and due to priority of the species epithet *aquamarina* they were reclassified in the species *Deleya aquamarina*. Meanwhile, members of the genus *Deleya* have been transferred to the genus *Halomonas*, and the new combinations *H. aquamarina*, *H. pacifica*, *H. cupida*, and *H. venusta* have been proposed for the marine *Alcaligenes* species (Dobson and Franzmann, 1996).

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Genus I. **Alcaligenes** *Castellani and Chalmers 1919, 936*[AL]

HANS-JÜRGEN BUSSE AND GEORG AULING

Al.ca.li' ge.nes. Arabic *al* the; Arabic n. *galiy* the ash of saltwort; French n. *alcali* alkali; Gr. v. *gennaio* to produce; M.L. masc. n. *Alcaligenes* alkali-producing (bacteria).

Rods or coccobacilli, 0.5–1.2 × 1.0–3.0 µm, usually occurring singly. Resting stages not known. **Gram negative**. **Motile** with one to nine **peritrichous** flagella. **Obligately aerobic**, possessing a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Some strains are capable of anaerobic respiration in the presence of nitrate or nitrite. Optimal growth temperature: 20–37°C. Colonies on nutrient agar are **nonpigmented**. **Oxidase positive**. **Catalase positive**. Indole not produced. Cellulose, esculin, gelatin, and DNA usually not hydrolyzed. **Chemoorganotrophic, using a variety of organic acids and amino acids as carbon sources. Alkali produced** from several organic salts and amides. Carbohydrates usually not utilized. **Characteristic fatty acids are $C_{17:0\ cyclo}$, $C_{16:0}$, $C_{14:0\ 3OH}$, $C_{16:1}$, and $C_{12:0\ 2OH}$. Ubiquinone Q-8. Polyamine patterns with the predominant compound putrescine and the unusual diamine 2-hydroxyputrescine.** Isolated from water, soil, and clinical specimens such as blood, spinal fluid, pleural fluid, peritoneal fluid, pus, urine, stools, and swabs of eyes, ears, and pharynxes. Frequently found in unster-

ilized distilled water and in chlorhexidine solutions in hospitals. Occasionally causing opportunistic infections in humans.

The mol% G + C of the DNA is: 56–60.

Type species: **Alcaligenes faecalis** Castellani and Chalmers 1919, 936[AL] (*Achromobacter arsenoxydans-tres* Turner 1954, 475; "*Pseudomonas odorans*" (Málek and Kazdová-Košiskova 1946; Málek, Radochová and Lysenko 1963, 353; "*Alcaligenes odorans* var. viridans" Mitchell and Clarke 1965, 347.

FURTHER DESCRIPTIVE INFORMATION

For a broad and an extensive view the reader is referred to the excellent treatment of the genus by Kersters and De Ley (1984b) except for those characteristics that became available since the first edition of *Bergey's Manual of Systematic Bacteriology*. However, since the Kersters and De Ley publication, many of the xenobiotic-degrading soil bacteria, especially some of the strains well known for plasmid-encoded pathways that were originally allocated to *Alcaligenes* species, have been transferred to other genera

(Busse and Auling, 1992, cf. also the family *Alcaligenaceae* in this volume).

Dimethyl disulfide-producing bacteria have been isolated from activated sludge and some of them were phenotypically allocated to the genus *Alcaligenes* (Tomita et al., 1987). In the same study pure cultures from the authentic genus *Alcaligenes* were also shown to produce dimethyl disulfide from DL-methionine and *S*-methyl-L-cysteine. More recently, an *Alcaligenes*-like dimethyl sulfide-producing marine isolate, phenotypically identified (Vitek), was shown to contain a dimethylsulfoniopropion-

ate lyase (de Souza and Yoch, 1995). This enzyme was purified and the authors argued that the K_m value and other properties observed may reflect the greater potential of facultative anaerobes over aerobes to metabolize lower levels of dimethylsulfoniopropionate in anoxic zones of seawater and salt marshes.

FURTHER READING

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List of species of the genus Alcaligenes

1. **Alcaligenes faecalis** Castellani and Chalmers 1919, 936[AL] (*Achromobacter arsenoxydans-tres* Turner 1954, 475; "*Pseudomonas odorans*" (Málek and Kazdová-Košiskova 1946; Málek, Radochová and Lysenko 1963, 353; "*Alcaligenes odorans* var. viridans" Mitchell and Clarke 1965, 347.)

   fae.ca' lis. L. n. *faex, faecis* dregs; M.L. adj. *faecalis* fecal.

   The species *Alcaligenes faecalis* is subdivided into the two subspecies, *A. faecalis* subsp. *faecalis* and *A. faecalis* subsp. *parafaecalis.*

   The mol% G + C of the DNA is: 56–60 (T_m, Bd).

   a. **Alcaligenes faecalis** *subsp.* **faecalis** Castellani and Chalmers 1919, 936[AL]

      The description of *Alcaligenes faecalis* subsp. *faecalis* is based on that given by Kersters and De Ley (1984b) for *A. faecalis*. This subspecies is the type subspecies of *Alcaligenes faecalis* and contains the type strain of the species. The morphological characteristics are as described for the genus. Colonies on nutrient agar are nonpigmented to grayish white, translucent to opaque, flat to low convex, margin usually entire, usually smooth, sometimes dull or rough. Most strains form colonies with a thin, spreading irregular edge. Some strains previously named "*A. odorans*" (Málek and Kazdová-Koziškova, 1946; Málek et al., 1963), produce a characteristic aromatic fruity odor and/or a green discoloration on blood agar. Those strains producing the latter characteristic were previously named "*A. odorans* var. viridans" (Mitchell and Clarke, 1965).

      Physiological and nutritional characteristics of *A. faecalis* are presented in Table BXII.β.29 of the chapter *Alcaligenaceae* and Tables BXII.β.30 and BXII.β.31 of this chapter. Carbohydrates are not utilized as sole carbon sources. Good growth is obtained on several organic acids and amino acids. Instead of using a monoamine oxidase, *Alcaligenes faecalis* initiates catabolism of aromatic amines alternatively by an aromatic amine dehydrogenase that is structurally similar to methylamine dehydrogenase and possesses the same tryptophan tryptophylquinone prosthetic group as the latter (Govindaraj et al., 1994). Chemolithotrophic growth using hydrogen gas has not been demonstrated.

      Nitrite, but not nitrate, is reduced. Anaerobic respiration with nitrite, but not with nitrate, as a sole electron acceptor is possible for most strains. Some strains oxidize arsenite. *Alcaligenes faecalis* is among the bacterial taxa that are able to combine aerobic denitrification and heterotrophic nitrification (van Niel et al., 1992; Anderson et al., 1993) and that may contribute to biogenic emis-

sions of NO and N_2O into the atmosphere even when growing logarithmically (Papen et al., 1989; Otte et al., 1996). Since *Alcaligenes faecalis* is commonly found in soil, water, and waste water treatment plants, bacteria of this species, enriched by the denitrification process of activated sludge, may be responsible for significant trace gas emissions of suboptimally functioning systems of waste water treatment. Among a large collection of isolates from biofilters for off-gas treatment of animal-rendering plant emissions, 21 bacteria were allocated by a polyphasic taxonomic approach to either *Alcaligenes faecalis* or a new taxon (cluster J) within the family *Alcaligenaceae* (Ahrens et al., 1997).

Degradation of microbial reserve polymers such as poly-(3-hydroxybutyrate) (PHB) is known to occur in marine environments and a PHB depolymerase was purified and characterized from a marine bacterium, *Alcaligenes faecalis* strain AE122 (Kita et al., 1995). From activated sludge an *Alcaligenes faecalis* strain, T1, was isolated that can hydrolyze not only water-insoluble PHB but also water-soluble D(−)-3-hydroxybutyrate oligomeric esters. The PHB depolymerase of this strain was cloned, and the structure and function of its three domains (catalytic, substrate-binding, and fibronectin type-like) were studied (Saito et al., 1989; Nojiri and Saito, 1997). Resting cells of *Alcaligenes faecalis* ATCC 8750 have been described as producing *R*-(−)-mandelic acid from mandelonitrile for synthesis of semisynthetic cephalosporins (Yamamoto et al., 1991). D-Aminoacylase with high stereospecificity has been purified from *Alcaligenes faecalis* strain DA1 (Yang et al., 1991b).

A. faecalis subsp. *faecalis* has a fatty acid profile consisting of $C_{17:0\ cyclo}$, $C_{16:0}$, $C_{14:0\ 3OH}$, $C_{18:1}$, $C_{12:0\ 2OH}$, $C_{16:1\ \omega7c}$, $C_{12:0}$, $C_{19:0\ cyclo}$, $C_{14:0}$, and an unknown fatty acid (Vandamme et al., 1995c); phosphatidylethanolamine, phosphatidylglycerol, and an ornithine lipid (Yabuuchi et al., 1995), and an ubiquinone Q-8 (Lipski et al., 1992) are present; the polyamine pattern contains the predominant compound putrescine and the unusual 2-hydroxyputrescine (Busse and Auling, 1988).

Isolated from soil, water, feces, urine, blood, sputum, wounds, pleural fluid, nematodes, and insects.

The mol% G + C of the DNA is: 55.9–59.4 (T_m, Bd) (De Ley et al., 1970b; Pichinoty et al., 1978).

Type strain: ATCC 8750, CCM 1052, CCUG 1325, CIP 60.80, DSM 30030, IAM 12586, IFO 13111, IMET 10443, JCM 1472, LMG 1229, NCDO 868, NCIB 8156.

GenBank accession number (16S rRNA): M22508.

TABLE BXII.β.30. Differentiating characteristics of *Alcaligenes* and *Achromobacter*[a, b]

Characteristic	*Alcaligenes faecalis*	*Alcaligenes defragrans*[c]	*Alcaligenes latus*	*Achromobacter xylosoxidans* subsp. *denitrificans*	*Achromobacter xylosoxidans* subsp. *xylosoxidans*	*Achromobacter piechaudii*	*Achromobacter ruhlandii*
Gram reaction	−	−	−	−	−	−	−
Peritrichous flagella	+	+	+	+	+	+	+
Oxidase, catalase	+		+	+	+	+	+
Nitrate reduced to nitrite	−	+	+	[+]	+	+	+
Nitrite reduction	+	+	−	[+]	+	−	−
Nitrate respiration	−	+	−	[+]	+	−	
Nitrite respiration	+	+	−	[+]	+	−	
Chemolithotrophic growth with H$_2$	−		+	−	−	−	+
Hydrolysis of:							
Gelatin, starch, Tween 80	−		+	−	+	−	−
Urease	−			−	−	−	−
Acid from:							
D-Glucose	−			−	+	−	+
D-Xylose	−			−	+	−	+
Carbon source for growth:[d]							
D-Glucose	−	−	+	−	+		+
D-Xylose	−	−	−	−	+		+
D-Fructose	−	−	+	−	d		
D-Arabinose	−	−		−	d		+
D-Mannose	−	−		−	d		−
Adipate, pimelate	−	−	−	+	+	+	+
D-Gluconate	−	d[e]	+	[+]	+	+[f]	+
Sucrose	−	−[g]	+	−	−		
Trehalose	−	−[g]	d	−	−		
D-Arabitol	−		d	−	−		
Sebacate	−	−	d	+	+		+
Suberate	−	−	+	+	+		+
meso-Tartrate	−		−	+	+		+
D-Tartrate	−		+	d	d		−[f]
D-Malate	−	+[e]	−	+	+		+[f]
Acetate	+	+	−	+	+	+	
Valerate, isovalerate	d		−	d	+	+	
Itaconate	−	−	+	+	+	+	+
Mesaconate	−		−	d	+	+	+
Glycerol	−		+	d	d		
β-Alanine	−	+[e]	+	d	d		+
Propionate	+	+	d	+	+		
Citrate	+		d	+	+		+
N-Acetylglucosamine	−			−	−		−
Pimelate	−	−	−	+	+	+	
Pimelate	−	−	−	+	+	+	
5-Ketogluconate, esculin	−			−	−	−	
D-Lyxose, L-xylose, inulin, D-tagatose, melezitose, L-fucose, arbutin, gentiobiose, turanose, L-arabitol, adonitol, glycogen, melibiose, amygdalin, spermine, histamine, ethanolamine, benzylamine, pentylamine	−			−	−		
Maltose, 2-ketogluconate, butylamine, betaine, creatine, sarcosine, *p*-hydroxybenzoate	−		+	−	−		
Butyrate, succinate, fumarate	+	+	+	+	+		
D- and L-α-alanine, L-glutamate	+	+[e]	+	+	+		
L-Malate	+	+[e]	+	+	+	+[f]	+[f]

(*continued*)

TABLE BXII.β.30. *(cont.)*

Characteristic	*Alcaligenes faecalis*	*Alcaligenes defragrans*[c]	*Alcaligenes latus*	*Achromobacter xylosoxidans* subsp. *denitrificans*	*Achromobacter xylosoxidans* subsp. *xylosoxidans*	*Achromobacter piechaudii*	*Achromobacter ruhlandii*
DL-Lactate, DL-β-hydroxybutyrate, L-proline, L-aspartate	+		+	+	+		
Isobutyrate	d		+	d	+		
Heptanoate	d	+	−	d	d		
Caproate, caprylate, pelargonate, maleate	d		−	d	d		−
Glycine	d		−	d	d		+
Benzoate	d	−	−	d	d		
Caprate	+		−	d	+[c]		+[a]
Malonate	+		+	d	−		−
Glutarate	d	+	−	+	+		
α-Ketoglutarate	d		−	+	+		
Azelate	−			+	+		+
Glycolate	+		d	d	−		
DL-Glycerate, L-ornithine	d		+	d	d		
L-Tartrate	−	−	d	d	−		
Pyruvate	d	+	−	+	d		
Aconitate	d	+	+	+			+[j]
Citraconate	d		−	d	+		
m-Hydroxybenzoate	−		+	d	−		+
L-Mandelate	d		+	d	−		
Phenylacetate	+		+	d	+	+	+
L-Leucine	+		+	d	+		−
Quinate			+	−			−
L-Serine	−		+	d	+		+
L-Threonine	d		+	+	+		−
L-Isoleucine	+		d	d	+		−
L-Valine	d	+[e]		d	+		
L-Lysine	d		−	d	d		+
L-Arginine	−	d[e]	−	−			
L-Citrulline	−		+	d	d		+[f]
γ-Aminobutyrate	−		+	d	d		+
DL-Norleucine	+		−	d	d		−[e]
L-Tryptophan	+		−	d	d		
δ-Aminovalerate	−		−	d	d		
L-Histidine	d		−	d	+		+
L-Tyrosine	d		d	d	d		
L-Phenylalanine	+	−[e]	−	+	+		
L-Cysteine, tryptamine, DL-kynurenine	d			d	d		
L-Methionine	d			−	−		
Kynurenate, putrescine			−				
Acetamide	d		−	d	−		

[a]Symbols: +, 90% or more of the strains are positive; [+], 80% or more of the strains are positive; d, 11–79% of the strains are positive; −, <11% of strains positive.

[b]Data from Yamasato et al. (1982), Kersters and DeLey (1984b), Kiredjian et al. (1986), Vandamme et al. (1996a), Foss et al. (1998a), Yabuuchi et al. (1998a).

[c]Utilization of carbon sources was measured under denitrifying conditions (Yamasato et al., 1982).

[d]The species *A. faecalis*, *A. xylosoxidans* subsp. *denitrificans*, *A. xylosoxidans* subsp. *xylosoxidans*, *A. defragrans*, and *A. latus* do not utilize any of the following carbon sources: D-ribose or meso-inositol (no data available for *A. piechaudii* and *A. ruhlandii*). The species *A. faecalis*, *A. xylosoxidans* subsp. *denitrificans*, *A. xylosoxidans* subsp. *xylosoxidans*, *A. ruhlandii*, and *A. latus* do not utilize any of the following carbon sources: L-arabinose or D-mannitol (no data available for *A. defragrans* and *A. piechaudii*). The species *A. faecalis*, *A. xylosoxidans* subsp. *denitrificans*, *A. xylosoxidans* subsp. *xylosoxidans*, and *A. latus* do not utilize any of the following carbon sources: L-arabinose, D-mannitol, D-galactose, L-sorbose, raffinose, dulcitol, D-fucose, L-rhamnose, cellobiose, lactose, salicin, meso-erythritol, sorbitol, oxalate, levulinate, o-hydroxybenzoate, D-mandelate, phthalate, L-arginine, m-aminobenzoate, or p-aminobenzoate (no data available for *A. defragrans*, *A. ruhlandii*, and *A. piechaudii*).

[e]The mechanism of compound use not specified (Foss et al., 1998a).

[f]D-(−)-Tartrate, DL-malic acid, *trans*-aconitate, and DL-citrulline were subjected to assimilation tests (Yamasato et al., 1982).

[g]The D-isomer of the respective carbon source was not used (Foss et al., 1998a).

Additional Remarks: Other representative culture collection strains belonging to this taxon: ATCC 15554, CCEB 554, NCTC 10416, DSM 30033; LMG 1230; CIP 71.8 (formerly "*A. odorans*"); ATCC 19209, NCTC 10388, Burchill 1 (formerly "*A. odorans* var. viridans"); NCIB 8687; LMG 3368; LMG 3394 (formerly "*Achromobacter arsenoxidans*").

b. **Alcaligenes faecalis** *subsp.* **parafaecalis** Schroll, Busse, Parrer, Rölleke, Lubitz and Denner 2001b, 1619^VP (Effective publication: Schroll, Busse, Parrer, Rölleke, Lu-

TABLE BXII.β.31. Physiological and biochemical traits distinguishing *Alcaligenes faecalis* subsp. *faecalis* and *Alcaligenes faecalis* subsp. *parafaecalis*[a,b]

Characteristic	A. faecalis subsp. faecalis (n = 2)	A. faecalis subsp. parafaecalis (n = 1)
Nitrite reduction	+	−
Gelatin liquefaction (37°C)	−	+
Assimilation of:[c]		
L-Histidine	+	−
L-Tryptophan	+	−
Benzoate	+	−
Gentisate	−	+
Biolog reactions:[d]		
L-Serine	+	−
L-Ornithine	+	−
L-Histidine	+	−
Aerobic growth at 42°C	+	−
Growth in the presence of 7% NaCl	+	−
Diagnostic polyamine content:		
2-hydroxyputrescine[e]	1.7–8.9	tr

[a]Symbols: +, positive reaction; −, no reaction.

[b]Data from Busse and Auling (1988), Ahrens et al. (1997), and Schroll et al. (2001a).

[c]Estimated by API Biotype 100 test.

[d]Estimated by Biolog GN system.

[e]mol/g dry weight; tr = trace amounts (<0.1 mol/g).

bitz and Denner 2001a, 41.)

para.fae.ca′ lis. Gr. prep. *para* along side of, resembling; M.L. adj. *faecalis* specific epithet; M.L. *parafaecalis* intended to mean alongside of the species *A. faecalis.*

The description of this subspecies is taken from Schroll et al. (2001a). Cells are Gram negative, rod shaped, 0.75–1.0 × 1.5–3.0 μm, nonsporeforming, and motile. Colonies on standard bacteriological media are circular, entire, low convex, and smooth with a thin spreading irregular edge; diameter is up to 2.0 mm after 1 d incubation at 37°C. The following tests were positive: oxidase activity, catalase activity, alkalization, growth on MacConkey agar, citrate utilization, acetoin production, gelatin liquefaction at 37°C, assimilation of gentisate; enzymatic activities as follows: L-alanine aminopeptidase, alkaline phosphatase, esterase (C_4), leucine arylamidase, valine arylamidase, acid phosphatase, weak reactions for esterase lipase (C_8), lipase (C_{14}), naphthol-AS-BI-phosphohydrolase. No growth at 42°C or on MacConkey agar. Acid is not produced in Hugh/Leifson's glucose O-F medium, and L-histidine, L-tryptophan, and benzoate are not assimilated. Lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, indole production, H_2S production, nitrate reduction, nitrite reduction, urease activity, acetoin production, gelatin liquefaction at 28°C, hydrolysis of Tween 80 and casein, DNase activity, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are negative. The following compounds are assimilated (as tested by growth in M9 medium): acetate, propionate, butyrate, valerate, heptanoate, γ-hydroxybutyric acid, γ-butyrolactone, L-lactate, citrate, ethanol. By using the Biolog GN system the following reactions are positive: Tween 40, Tween 80, meth-

ylpyruvate, mono-methyl pyruvate, acetic acid, *cis*-aconitic acid, citric acid, formic acid; α-, β-, and γ-hydroxybutyric acid, *p*-hydroxyphenyl acetic acid, α-ketobutyric acid, D,L-lactic acid, malonic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid, alaninamide, D-alanine, L-alanine, L-glutamic acid, L-leucine, L-phenylalanine, L-proline, urocanic acid, and phenylethylamine; weak reactions are observed for glycogen, L-alanylglycine, L-asparagine, L-aspartic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, D-serine, L-threonine, and inosine. The following compounds are not assimilated (as tested by growth in M9 medium): 1,4 butandiol, 2,3 butandiol, methanol, 1-butanol, 2-butanol, 1-hexanol, glycerol, acetone, and tartric acid. Using the Biolog GN system, the following reactions are negative: α-cyclodextrin, dextrin, N-acetylglucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, *i*-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *meso*-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, itaconic acid, α-ketoglutaric acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-pyroglutamic acid, L-serine, D,L-carnitine, γ-amino butyric acid, uridine, thymidine, putrescine, 2-amino ethanol, 2,3 butanediol, glycerol, D,L-α-glycerol phosphate, glucose-1-phosphate, and glucose-6-phosphate.

A. faecalis subsp. *parafaecalis* has a fatty acid profile consisting of $C_{16:0}$, $C_{16:1}$, $C_{17:0\ cyclo}$, $C_{18:1\ \omega7c/9t}$, $C_{14:0\ 3OH}$, $C_{14:0}$, $C_{12:0\ 2OH}$, $C_{12:0}$, $C_{18:0}$, $C_{15:0}$, and $C_{17:0}$; a ubiquinone Q-8; and a polyamine pattern with putrescine as the predominant compound and only trace amounts of the *Betaproteobacteria*-specific polyamine 2-hydroxyputrescine.

Isolated from water (garden pond, Lower Austria; Austria).

The mol% G + C of the DNA is: 56 (T_m).

Type strain: G, CIP 106866, DSM 13975.

GenBank accession number (16S rRNA): AJ242986.

2. **Alcaligenes defragrans** Foss, Heyen and Harder 1998b, 1083[VP] (Effective publication: Foss, Heyen and Harder 1998a, 243.)

de.fra.grans. L. prep. *de* away, from; L. adj. *fragrans* sweet scented; M.L. defragrans to annihilate fragrance, referring to the capacity to degrade monoterpenes.

The morphological characteristics are as described for the genus. The facultatively anaerobic chemoorganotrophic metabolism is strictly oxidative. Oxygen, nitrate, nitrite, or dinitrogen oxide can serve as electron acceptor. Under denitrifying conditions, acetate, propionate, butyrate, hexanoate, heptanoate, octanoate, pyruvate, malate, succinate, fumarate, 3-methylbutyrate, glutarate, glutamate, alanine, valine, ethanol, and the monoterpenes (+)-ρ-menth-1-ene, (+)-limonene, (−)-α-phellandrene, α-terpinene, γ-terpinene, (+)-sabinene, (+)-2-carene, (+)-3-carene, (−)-α-pinene, (−)-β-pinene, terpinolene, (+)-α-terpineol, and

(+)-terpinen-4-ol are used as carbon and energy sources. The majority of strains grow on myrcene, arginine, and gluconate. Under denitrifying conditions no growth occurs on D-glucose, D-fructose, D-sorbitol, *meso*-inositol, D-ribose, D-arabinose, D-xylose, D-saccharose, D-trehalose, D-cellobiose, ascorbate, phenylalanine, formate, L-tartrate, adipate, pimelate, suberate, sebacate, itaconate, methanol, cyclohexanol, cyclohexane-1,2-diol, cyclohexane-1,4-diol, decane, hexadecane, heptamethylnonane, cyclohexane, benzoate, toluene, 2,6-dimethyloctane, 3,7-dimethyl-1-octene, (−)-β-citronellene, 3,7-dimethyloctanol-1, (−)-β-citronellol, geraniol, nerol, linalol (+)-*trans*-isolimonene, ρ-cymene, (+)-perilla alcohol, (−)-carveol, (+)-dihydrocarveol, menthol, menthone, (+)-isopulegol, (+)-isomenthol, (+)-pulegone, (−)-carvone, (+)-dihydrocarvone, (−)-*trans*-pinane, eucalyptol, (−)-*cis*-myrtanol, (+)-*trans*-myrtanol, (−)-myrtenol, (+)-isopinocampheol, (−)-borneol, (+)-fenchol, (α+β)-thujone, (+)-fenchone, (−)-verbenone, (+)-camphor, and pine needle oil.

When grown on monoterpene and nitrogen, no vitamins are needed, and growth occurs at 15–40°C in the range of pH 5.9–8.4; pH-optimum is at 6.3–7.8.

The whole cell fatty acid profile is strongly dependent on the culture conditions. When grown under denitrifying conditions on monoterpene, the fatty acid profile is characterized by the predominant acids $C_{16:0}$ and $C_{17:0\ cyclo}$ and varying amounts of dodecanal, $C_{12:0}$, $C_{14:0}$, $C_{14:0\ 3OH}$, $C_{15:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{19:0\ cyclo}$. When acetate is the only carbon and energy source $C_{16:1}$, $C_{16:0}$, $C_{17:0\ cyclo}$, and $C_{18:1}$ are the predominant fatty acids.

The species *Alcaligenes defragrans* was proposed by Foss et al. (1998a) to encompass four monoterpenes degrading strains. 16S rDNA sequence comparisons indicate that *A. defragrans* is more closely related to *Bordetella* and *Achromobacter* than *A. faecalis*. This relatedness is also reflected by its DNA base ratio, which is in the range of *Achromobacter* and *Bordetella.*

Isolated from activated sludge and a forest ditch.

The mol% G + C of the DNA is: 66.9 (HPLC).

Type strain: 54Pin, DSM12141.

GenBank accession number (16S rRNA): AJ005447.

Additional Remarks: Other representative culture collection strains belonging to this taxon: DSM 12142 (62Car), DSM 12143 (65Phen), DSM 12144 (51Men) (*Alcaligenes defragrans*).

Species Incertae Sedis

1. **Alcaligenes latus** Palleroni and Palleroni 1978, 423[AL]
   la' tus. L. adj. *latus* broad.

   The cells are short to coccoid rods, 1.2–1.4 × 1.6–2.4 μm, occurring singly, in pairs, or in short chains. Gram negative. Motile by means of 5–10 peritrichous flagella. The cells are frequently heavily granulated. Under autotrophic growth conditions on a solid mineral medium colonies are round, grayish pink, and opaque (Palleroni and Palleroni, 1978). Colonies are wrinkled in fresh isolates but can become smooth upon subcultivation.

   Facultatively chemolithotrophic in an atmosphere containing hydrogen, oxygen, and carbon dioxide. Can grow with dinitrogen as sole nitrogen source (H.G. Schlegel, personal communication). Physiological and nutritional characteristics are given in Table BXII.β.29 of *Alcaligenaceae* and Table BXII.β.30 of this chapter. Optimal growth temperature is about 35°C. A membrane-bound hydrogenase, but no soluble, NAD-reducing hydrogenase has been found in three strains of *A. latus* (Palleroni and Palleroni, 1978). *meta*-Hydroxybenzoate is metabolized via the gentisate pathway and protocatechuate is degraded by *meta*-cleavage when grown on *p*-hydroxybenzoate or quinate.

   Three strains were isolated from soil (Palleroni and Palleroni, 1978). The fatty acid profile is characterized by the following compounds: $C_{16:1}$, $C_{18:1}$, $C_{16:0}$, $C_{18:0}$, $C_{14:0}$, $C_{12:0}$, $C_{17:0\ cyclo}$, $C_{17:0}$, $C_{15:0}$, $C_{10:0\ 3OH}$, and $C_{14:1}$ and the major respiratory quinone is ubiquinone Q-8 (Oyaizu-Masuchi and Komagata, 1988).

   The mol% G + C of the DNA is: 69.1–71.1 (Bd, T_m) (Palleroni and Palleroni, 1978; Kersters and De Ley, 1984b).

   Type strain: Palleroni H-4, ATCC 29712, CIP 10345, DSM 1122.

   GenBank accession number (16S rRNA): D88007.

   The species *Alcaligenes latus* was proposed by Palleroni and Palleroni (1978) to encompass hydrogen oxidizing, peritrichously flagellated bacteria. DNA–rRNA hybridization studies revealed that this species is most closely related to *Rubrivivax gelatinosus* and *Leptothrix discophora* (Willems et al., 1991b), and distant from the family *Alcaligenaceae*. Thus, *A. latus* cannot be considered as a member of the genus or even of the family. It is most likely that *A. latus* is a member of a genus that has not yet been described. The species description of *A. latus* is a revised version of that given by Kersters and De Ley (1984b).

Genus II. **Achromobacter** *Yabuuchi and Yano 1981, 477*[VP] *emend. Yabuuchi, Kawamura, Kosako and Ezaki 1998a, 1083*

HANS-JÜRGEN BUSSE AND GEORG AULING

A.chro.mo.bac' ter. Gr. adj. *achromus* colorless; M.L. n. *bacter* the masc. equivalent of Gr. neut. n. *bactrum* a rod or staff; M.L. masc. n. *Achromobacter* colorless rodlet.

Straight rods, 0.8–1.2 × 2.5–3.0 μm with rounded ends. **Gram negative**. Nonsporeforming. **Motile** with 1–20 sheathed flagella arranged **peritrichously**. **Obligately aerobic** and nonfermentative. Some strains are capable of anaerobic respiration with nitrate as the electron acceptor. They perform nitrate respiration combined with nitrite and nitrous oxide respiration. Some strains are facultative lithoautotrophic hydrogen-oxidizers. **Oxidase positive**. **Catalase positive**. Urease, DNase, phenylalanine deaminase, lysine and ornithine decarboxylase, arginine dihydrolase, and gelatinase negative. Nonhalophilic, nonhemolytic, nonpigmented. **Chemoorganotrophic, using a variety of organic acids and amino acids as carbon sources.** Carbohydrates usually not utilized. *Achromobacter xylosoxidans* subsp. *xylosoxidans* and *Achromobacter ruhlandii* utilize D-glucose as sole carbon source and produce acid from D-glucose, D-arabinose, and D-xylose. **Those strains analyzed contain the characteristic fatty acids $C_{17:0\ cyclo}$,**

$C_{16:0}$, $C_{14:0\ 3OH}$, $C_{16:1}$, $C_{12:0\ 2OH}$, and **ubiquinone Q-8**. The poly-amine patterns display **putrescine** as the major compound and contain the unusual diamine **2-hydroxyputrescine**.

Isolated from water, soil; also from hospital environment and human clinical specimens with pathological significance or as contaminants.

The mol% G + C of the DNA is: 65–68.

Type species: **Achromobacter xylosoxidans** Yabuuchi and Yano 1981, 477.

FURTHER READING

De Ley, J., P. Segers, K. Kersters, W. Mannheim and A. Lievens. 1986. Intrageneric and intergeneric similarities of the *Bordetella* ribosomal ribonucleic acid cistrons: proposal for a new family, *Alcaligenaceae*. Int. J. Syst. Bacteriol. *36*: 405–414.

List of species of the genus Achromobacter

1. **Achromobacter xylosoxidans** (ex Yabuuchi and Ohyama 1971) Yabuuchi and Yano 1981, 477^{VP} emend. Yabuuchi, Kawamura, Kosako and Ezaki 1998a, 436.

   xy.los.ox′.i.dans. Gr. n. *xylon* wood, xylose, wood sugar; Gr. adj. *oxys* sharp, acid; L. part. *dans* giving; M.L. pres part. *oxydans* acid-giving, oxidizing; M.L. *xylosoxydans* oxidizing xylose.

   The description is taken from Yabuuchi et al. (1998a). The morphological characteristics are as described for the genus. Colonies on nutrient agar are circular, nonpigmented to grayish white, translucent to opaque, flat to convex, usually smooth, sometimes dull or rough, margin usually entire. On heart infusion agar colonies are 1 mm in diameter, low convex with entire margin, moist, and with a glistening surface. Grows anaerobically in the presence of nitrate or nitrite by denitrification. In the API 20NE test citrate, adipic acid, DL-malic acid, and phenylacetate are assimilated. Assimilation of gluconate, capric acid, and glucose is variable. L-arabinose, D-mannitol, D-mannose, maltose, and N-acetyl-D-glucosamine are not assimilated.

   The mol% G + C of the DNA is: 63.9–69.8 (Bd, T_m).

   a. **Achromobacter xylosoxidans** *subsp.* **xylosoxidans** (ex Yabuuchi and Ohyama 1971) Yabuuchi and Yano 1981, 477^{VP} (*Alcaligenes denitrificans* subsp. *xylosoxidans* (Yabuuchi and Yano 1981) Kersters and De Ley 1984b, 367; *Alcaligenes xylosoxidans* subsp. *xylosoxidans* (Yabuuchi and Yano 1981) Kiredjian, Holmes, Kersters, Guilvout and De Ley 1986, 285.)

      If not stated otherwise, the description is taken from Kersters and De Ley (1984b) and Yabuuchi et al. (1998a). Nearly all strains utilize D-glucose, D-xylose, D-gluconate, adipate, and pimelate as sole carbon sources and characteristically form acid from D-xylose in the O/F medium of Hugh and Leifson (1953). Further characteristics are shown in Table BXII.β.30.

      A plasmid-encoded resistance by two distinct nickel resistance loci (high- and low-level) against nickel, cobalt, and cadmium has been described for strain *Achromobacter xylosoxidans* 31A (Schmidt et al., 1991; Schmidt and Schlegel, 1994), originally isolated from a copper galvanization tank. Maintenance of the biodegradation capacities of *A. xylosoxidans* and other Gram-negative aerobic bacteria containing large plasmids during long-term preservation was studied and protective agents have been described (Lang and Malik, 1996).

      Mostly isolated from clinical specimens such as blood, sputum, wounds, purulent ear discharge, spinal fluid, cerebral tissue, urine, feces, and, in a few cases, also from disinfection solutions.

      The fatty acid profile is characterized by the following compounds: $C_{16:0}$, $C_{17:0\ cyclo}$, $C_{14:0\ 3OH}$, $C_{16:1}$, $C_{14:0\ 2OH}$, $C_{12:0\ 2OH}$, $C_{16:0\ 2OH}$, $C_{18:0}$, $C_{14:0}$, and $C_{19:0\ cyclo}$ (Vandamme et al., 1995c). Phosphatidylethanolamine, phosphatidyl-glycerol, and diphosphadidylglycerol are the major compounds in the polar lipid profile (Yabuuchi et al., 1974). The polyamine pattern contains two major compounds: putrescine and the unusual 2-hydroxyputrescine (Busse and Auling, 1988).

      The mol% G + C of the DNA is: 66.9–69.8 (T_m) (De Ley et al., 1970b; Yabuuchi et al., 1974; Holmes et al., 1977b).

      Type strain: Hugh 2838, ATCC 27061, CIP 71.32, Yabuuchi KM 543, NCTC 10807.

      GenBank accession number (16S rRNA): X59163, D88005.

      Additional Remarks: The type strain was proposed as the type strain for *Achromobacter xylosoxidans* by Yabuuchi and Ohyama (1971). Other representative culture collection strains belonging to this taxon: CIP 58.72 and CIP 61.20 (formerly *A. denitrificans*). In the near future Coyne et al. will propose the reclassification of *Alcaligenes denitrificans* as *Achromobacter denitrificans* (Int. J. Syst. Evol. Microbiol., in press, 2003).

   b. **Achromobacter xylosoxidans** *subsp.* **denitrificans** (Rüger and Tan 1983) Yabuuchi, Kawamura, Kosako and Ezaki 1998b, 1083^{VP} (Effective publication: Yabuuchi, Kawamura, Kosako and Ezaki 1998a, 436) (*Alcaligenes denitrificans* Rüger and Tan 1983, 88; *Alcaligenes denitrificans* subsp. *denitrificans* Rüger and Tan 1983; *Alcaligenes xylosoxidans* subsp. *denitrificans* (Rüger and Tan 1983) Kiredjian, Holmes, Kersters, Guilvout and De Ley 1986, 285.)

      de.ni.tri′fi.cans. L. prep. *de* away, from; L. n. *nitrum* soda; M.L. n. *nitrum* nitrate; M.L. v. *denitrificans* to denitrify; M.L. pres. part. *denitrificans* denitrifying.

      If not stated otherwise the description is taken from Kersters and De Ley (1984b) and Yabuuchi et al. (1998a). Physiological and nutritional characteristics of the species are presented in Table BXII.β.29 of *Alcaligenaceae* and Table BXII.β.30 of this chapter. Most strains characteristically use *meso*-tartrate, itaconate, adipate, pimelate, and other dicarboxylic acids as sole carbon sources. Some strains are auxotrophic and require organic nitrogenous compounds for growth. Nitrates and nitrites are usually reduced. Most strains carry out an anaerobic respiration in the presence of nitrate and nitrite.

      The fatty acid profile is characterized by the following compounds: $C_{16:0}$, $C_{17:0\ cyclo}$, $C_{14:0\ 3OH}$, $C_{14:0}$, $C_{16:1}$, $C_{12:0\ 2OH}$, $C_{18:1}$, $C_{12:0}$, $C_{18:0}$, $C_{16:0\ 2OH}$, and $C_{19:0\ cyclo}$ (Vandamme et al., 1995c). Ubiquinone Q-8 is the predominant respiratory quinone (Lipski et al., 1992); the poly-

amine pattern contains two major compounds: putrescine and the unusual 2-hydroxyputrescine (Busse and Auling, 1988).

Isolated from soil, and a variety of clinical specimens such as feces, urine, blood, pleural fluid, purulent ear discharges, prostatic secretions, and throat swabs.

Strains of *A. xylosoxidans* subsp. *denitrificans* or *A. xylosoxidans* subsp. *xylosoxidans* have been isolated from soil and examined for presence of D-aminoacylase in order to produce D-amino acids from *N*-acetyl-DL-amino acids enzymatically. The presence of D-aminoacylase appeared not to be a general characteristic of members of the genus, although inducible D-aminoacylases with high stereospecificity and higher specific activity than those described from *Pseudomonas* and *Streptomyces* were found (Moriguchi and Ideta, 1988; Tsai et al., 1988). A novel cyanide-hydrolyzing enzyme from *A. xylosoxidans* subsp. *denitrificans* has been described (Ingvorsen et al., 1991). Recently a gene from *A. xylosoxidans* subsp. *denitrificans* has been analyzed that confers albicidin resistance by reversible antibiotic binding. This gene appears to be a useful candidate for transfer to plants to protect plastid DNA replication from inhibition by albicidin phytotoxins involved in sugarcane leaf scald disease (Basnayake and Birch, 1995). Uptake of chloro-substituted benzoic acids that are formed as dead-end metabolites by polychlorinated biphenyl-degrading microorganisms has been studied in *A. xylosoxidans* subsp. *denitrificans* (Miguez et al., 1995). *A. xylosoxidans* subsp. *denitrificans* was shown to mineralize the structurally related chlorinated phenoxy alkanoics, including 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2-(2-methyl-4-chlorophenoxy) propionic acid (mecoprop), which are among the most widely used herbicides to control broad-leafed weeds in cereal crops throughout the world. The strain described uses the *ortho*-pathway for biodegradation (Tett et al., 1994, 1997). Reductive dechlorination of 2,4-dichlorobenzoate and hydrolytic dehalogenation of 4-halo-substituted aromatics has been studied with *A. xylosoxidans* subsp. *denitrificans* NTB-1 (van der Tweel et al., 1987). Gene transfer of plasmid-encoded 2,2-dichloropropionate halidohydrolase from *A. xylosoxidans* subsp. *denitrificans* to other soil bacteria has been demonstrated using a soil microcosm (Brokamp and Schmidt, 1991). Recently the plasmid (pFL40)-encoded D,L-haloalkanoic acid dehydrogenase gene (*dhlIV*) was sequenced and expressed in members of the *Gammaproteobacteria* (Brokamp et al., 1997). Gas-phase methyl ethylketone biodegradation in a novel type of bioreactor was demonstrated with a bacterial consortium dominated by *A. xylosoxidans* subsp. *denitrificans*, run under non-axenic conditions (Agathos et al., 1997).

The mol% G + C of the DNA is: 63.9–68.9 (T_m, Bd) (De Ley et al., 1970b; Pichinoty et al., 1978).

Type strain: Hugh 12, ATCC 15173, CIP 77.15, DSM 30026, NCTC 8582.

GenBank accession number (16S rRNA): M22509.

Additional Remarks: Other representative culture collection strains belonging to this taxon are ATCC 13138, CIP 60.81 (*A. xylosoxidans* subsp. *denitrificans*).

2. **Achromobacter piechaudii** (Kiredjian, Holmes, Kersters, Guilvout and De Ley 1986) Yabuuchi, Kawamura, Kosako and Ezaki 1998b, 1083^{VP} (Effective publication: Yabuuchi, Kawamura, Kosako and Ezaki 1998a, 436) (*Alcaligenes piechaudii* Kiredjian, Holmes, Kersters, Guilvout and De Ley 1986, 285.)

pie.chau' di.i. M.L. gen. n. *piechaudii* from Piechaud, to honor M. Piechaud, a bacteriologist at the Institut Pasteur, Paris, France.

The description is taken from the original paper. Gram-negative, obligately aerobic, straight rods, 0.5–1.0 × 1.0–1.5 μm, which have parallel sides and rounded ends. Cells occur singly and are nonsporulating and noncapsulated. Cells are motile by means of two to eight peritrichous flagella, which have one or two wavelengths. At the optimal temperature (28–30°C) visible growth within 24 h. Liquid cultures (in nutrient broth medium) are uniformly turbid; cultures on trypticase soy agar produce small smooth colonies (diameter, 0.2 mm). Colonies are 1 mm in diameter after 48 h of incubation; no pigments or odor produced. Circular, smooth, entire colonies on nutrient agar after 24 h of growth. Colonies are nonhemolytic on 5% (v/v) horse blood agar. The organisms are nonfluorescent on King medium B and do not produce a brown diffusible melanin-like pigment on tyrosine agar. Growth without NaCl in peptone–water medium and at a maximum NaCl concentration of 7% (w/v). Alkaline reaction in glucose oxidation–fermentation medium. Catalase positive. Oxidase positive. Hydrolyzes tributyrin and tyrosine but not gelatin, Tween 20, Tween 80, esculin, or starch. Casein is not digested. Extracellular deoxyribonuclease or opalescence on lecithovitellin agar is not produced. Does not produce indole, urease, arginine desimidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, β-D-galactosidase (as determined by the *o*-nitrophenyl-β-D-galactopyranoside test) or phosphatase. Reduces nitrate but not nitrite; no anaerobic respiration in the presence of nitrate or nitrite. Produces hydrogen sulfide, as determined by the lead acetate paper method, but not as determined by the triple-sugar-iron agar method. Does not reduce thiosulfate. Grows on β-hydroxybutyrate (with production of lipid inclusion granules), on cetrimide, and on MacConkey agar. Produces alkali on Christensen citrate (as determined by the NCTC method; Kiredjian et al., 1986). Utilizes citrate (Simmons medium) as determined by the IP (Kiredjian et al., 1986) method but not as determined by the NCTC method; does not utilize malonate (as determined by the NCTC method). Oxidizes gluconate as determined by the IP method but not as determined by the NCTC method. Tolerates KCN at a concentration of 0.0075%. Does not produce 3-ketolactose. Does not reduce selenite and does not deaminate histidine, leucine, phenylalanine, or tryptophan. In minimal synthetic medium *n*-valerate, isovalerate, D-gluconate, mesaconate, and itaconate are assimilated. Does not assimilate carbohydrates in minimal synthetic medium. Produces acid in ammonium salt medium under aerobic conditions from ethanol. Does not produce acid in ammonium salt medium under aerobic conditions from D-glucose, adonitol, L-arabinose, D-cellobiose, dulcitol, D-fructose, glycerol, *m*-inositol, lactose, maltose, mannitol, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose, or D-xylose. Does not produce

acid from 10% (w/v) D-glucose or 10% (w/v) lactose. Does not produce acid or gas from D-glucose in peptone–water medium.

Hydrolyzes the following substrates (determined by using API ZYM galleries): L-leucyl-2-naphthylamide, glycyl-β-naphthylamide, L-aspartyl-β-naphthylamide, arginyl-β-naphthylamide, L-alanyl-β-naphthylamide, and L-leucyl-glycyl-β-naphthylamide. Does not hydrolyze the following substrates (determined by using API ZYM galleries): 2-naphthyl phosphate (at pH 8.5), 2-naphthyl caprylate, 2-naphthyl myristate, L-valyl-2-naphthylamide, L-cystyl-2-naphthylamide, N-benzoyl-DL-arginine-2-naphthylamide, N-glutaryl-phenylalanine-2-naphthylamide, naphthol-AS-B1-phosphodiamide, 6-bromo-2-naphthyl-α-D-galactopyranoside, 2-naphthyl-β-D-galactopyranoside, naphthol-AS-BI-β-D-glucuronic acid, 2-naphthyl-α-D-glucopyranoside, 6-bromo-2-naphthyl-β-D-glucopyranoside, 1-naphthyl-N-acetyl-β-D-glucosaminide, 6-bromo-2-naphthyl-α-D-mannopyranoside, 2-naphthyl-α-L-fucopyranoside, α-D-galactopyranoside, β-D-galactopyranoside, β-D-galactopyranoside-6-phosphate, α-L-arabinofuranoside, α-D-glucopyranoside, β-D-glucopyranoside, β-D-galacturonide, β-D-glucuronide, α-maltoside, β-maltoside, N-acetyl-α-D-glucosaminide, N-acetyl-β-D-glucosaminide, α-L-fucopyranoside, β-D-fucopyranoside, β-D-lactoside, α-D-mannopyranoside, β-D-mannopyranoside, α-D-xylopyranoside, β-D-xylopyranoside, L-tyrosyl-β-naphthylamide, L-phenylalanyl-β-naphthylamide, L-hydroxyprolyl-β-naphthylamide, L-histidyl-β-naphthylamide, N-benzoyl-L-leucyl-β-naphthylamide, S-benzoyl-L-cysteyl-β-naphthylamide, glycyl-L-prolyl-β-naphthylamide, N-carbobenzoxy-L-arginine-4-methoxy-β-naphthylamide hydrochloride, L-isoleucyl-β-naphthylamide, L-prolyl-β-naphthylamide hydrochloride, L-threonyl-β-naphthylamide, L-tryptophyl-β-naphthylamide and N-carbobenzoxy-glycyl-glycyl-L-arginine-β-naphthylamide.

Growth occurs at room temperature and 37°C but not at 5° or 42°C (optimal growth temperature 28–30°C). Although strains of *A. piechaudii* have been recovered mainly from human clinical material, few clinical details are available, so the clinical significance of the species is not yet determined.

The fatty acid profile is characterized by the following compounds: $C_{17:0\ cyclo}$, $C_{16:0}$, $C_{14:0\ 3OH}$, $C_{14:0}$, $C_{18:1}$, $C_{16:1}$, $C_{12:0\ 2OH}$, $C_{18:0}$, $C_{16:0\ 2OH}$, and $C_{19:0\ cyclo}$ (Vandamme et al., 1995c); the polyamine patterns contains two major compounds: putrescine and the unusual 2-hydroxyputrescine (Hamana and Takeuchi, 1998).

The mol% G + C of the DNA is: 64–65 (T_m).

Type strain: Hugh 366-5, CIP 60.75ATCC 43552, IAM 12591, LMG 1873.

GenBank accession number (16S rRNA): AB010841.

Additional Remarks: Other representative culture collection strains belonging to this taxon are LMG 6100 (CIP 55774), LMG 2828 (AB118), LMG 1861 (NCMB 1051), LMG 6102 (CL544/75), LMG 6101 (CL807/79), LMG 6103 (CL237/83).

3. **Achromobacter ruhlandii** (Aragno and Schlegel 1977) Yabuuchi, Kawamura, Kosako and Ezaki 1998b, 1083[VP] (Effective publication: Yabuuchi, Kawamura, Kosako and Ezaki 1998a, 436) (*Alcaligenes ruhlandii* (Packer and Vishniac 1955) Aragno and Schlegel 1977, 280.)

ruh.lan' di.i. M.L. gen. n. *ruhlandii* of Ruhland, named for the German microbiologist W. Ruhland, who studied the physiology of the hydrogen bacteria.

Cells are Gram-negative, nonsporeforming rods. Nonfermentative, nonhemolytic, and nonhalophilic. Growth occurs on MacConkey agar, in the presence of 5% NaCl, and at 41°C. Catalase and oxidase positive. Simmons' citrate medium is alkalized within 2 d and malonate broth within 3 d of incubation. Nitrate is reduced to nitrite. Gas is not produced in nitrate broth. Oxidatively acid is produced in OF medium from D-arabinose within 2 d and from L-arabinose, glucose, D-ribose, and D-xylose within 3 d of incubation. No acid is produced from fructose, galactose, cellobiose, lactose, maltose, melibiose, sucrose, trehalose, raffinose, melezitose, glycerol, adonitol, dulcitol, inositol, mannitol, sorbitol, salicin, and inulin. Acylamidase test is positive within 3 d of incubation. Urease, phenylalanine deaminase, and DNase are negative. Esculin, gelatin, starch, and Tween 80 are not hydrolyzed. Lysine and ornithine decarboxylase and arginine dihydrolase are negative. No pigments are produced. Glucose, adipic acid, capric acid, malic acid, citrate, gluconate, and phenylacetate are assimilated.

Isolated from soil. The polyamine pattern contains two major compounds: putrescine and the unusual 2-hydroxyputrescine (Busse and Auling, 1988).

The only available strain, *A. ruhlandii* ATCC 15749 (DSM 653), can grow autotrophically with hydrogen. Hydrogen-oxidizing strains similar to the only available strain of the species *A. ruhlandii* or other autohydrogenotrophic denitrifying members of the genus *Achromobacter* could not be isolated from autohydrogenotrophic pilot-reactors for denitrification of drinking water (Vanbrabant, et al., 1993; Auling and Luo, unpublished results).

The mol% G + C of the DNA is: 68.1 (T_m) (De Ley et al., 1986) and 67.7 (HPLC) (Yabuuchi et al., 1998a).

Type strain: ATCC 15749, DSM 653, IAM 12600.

GenBank accession number (16S rRNA): AB010840.

Other Organisms

For *Achromobacter* species (formerly assigned to the genus *Alcaligenes*) a significant environmental role on biodegradation of aromatic or halogenated compounds, even in the deep subsurface (Boivin-Jahns et al., 1995), is proposed by reports on isolation of strains, tentatively assigned to *A. xylosoxidans* (Ewers et al., 1990) and *A. denitrificans* (Weissenfels et al., 1990). The descriptive information is limited to the three species comprising the genus *Achromobacter*—*A. xylosoxidans*, *A. piechaudii*, and *A. ruhlandii*—and close relatives. Nevertheless, strains preliminarily identified as members of the genus *Alcaligenes* are still awaiting final taxonomic characterization, although published and named. Thus, care has to be taken when extrapolating the properties of such interesting strains to the whole species or even the genus.

The main phylogenetic aspects have been treated in the description of the family. DNA–DNA hybridizations indicated the emergence of an additional species in the neighborhood of *A. denitrificans* that may be centered around a strain O-1 able to degrade sulfonated aromatic compounds (Jahnke et al., 1990). The G + C content (66.1 mol%) of *Alcaligenes* sp. strain O-1

corresponds to that of the species. The DNA relatedness values obtained (51% to the type strain of *A. xylosoxidans* subsp. *xylosoxidans* and 40% to the type strain of *A. xylosoxidans* subsp. *denitrificans*) would justify a status as a separate species. Polyclonal antibodies raised against strain O-1 displayed the highest cross-reactivity with *A. xylosoxidans*. Thus, *Alcaligenes* O-1 can be considered as another species of the genus *Achromobacter*. *Alcaligenes* sp. strain O-1 contains a 117-MDa conjugative plasmid (pSAH) encoding mineralization of sulfonated aromatic compounds. The conjugative transfer of the toluene transposon Tn 4651 from *Pseudomonas putida* harboring plasmid pWW0, the archetypal TOL plasmid (Assinder and Williams, 1990), into plasmid pSAH extends the expression range of the TOL catabolic genes (degradation of 3-methylbenzoic acid) to the genus *Achromobacter* (Jahnke et al., 1993). A chlorobenzoate transposon (Tn5271) from the indigenous plasmid pBRC60 in *Alcaligenes* sp. strain BR60, isolated from runoff waters adjacent to a chlorobenzoate contaminated landfill, has been intensively studied genetically, although the taxonomic characterization of the host strain is still lacking (Windham et al., 1994).

The expression of xenobiotic-degrading genes using naphthalene as substrate has been investigated in *Alcaligenes* sp. (strain NP-Alk) versus *Pseudomonas putida* ATCC 17484, using these microorganisms as models for bioremediation of contaminated environments by either indigenous bacteria or strains released into an ecosystem (Guerin and Boyd, 1995).

The two biodegradative isolates, strain A3-C, able to degrade naphthalene sulfonic acid (Brilon et al., 1981), and strain B1, able to mineralize toluene sulfonic acid (Thurnheer et al., 1986), were identified as members of *A. denitrificans*, or classified within the genus *Alcaligenes* close to *A. denitrificans*, respectively (Busse et al., 1992).

Another strain (L6), from a collection of aerobic isolates proficient of 3-chlorobenzoate degradation under reduced oxygen partial pressures by metabolization via gentisate or the protocatechuate pathway, was allocated to the genus *Alcaligenes* by 16S rDNA sequencing (Krooneman et al., 1996). The authors demonstrated an increased oxygen affinity of strain L6 in studies with continuous culture, as is expected for organisms that are involved in metabolism of aromatic compounds and play an important role in determining the fate of haloaromatics at oxic-anoxic interfaces. Given their previous allocation to *A. denitrificans* or *A. xylosoxidans*, the strains A3-C, B1, and L6 are considered members of the genus *Achromobacter*.

Genus III. **Bordetella** *Moreno-López 1952, 178*[AL]

GARY N. SANDEN AND ROBBIN S. WEYANT

Bor.de.tel' la. M.L. dim ending *-ella;* M.L. fem. n. *Bordetella* named after Jules Bordet, who with O. Gengou first isolated the organism causing pertussis.

Minute coccobacillus, 0.2–0.5 µm in diameter and 0.5–2.0 µm in length, often bipolar stained, and arranged singly or in pairs, more rarely in chains. Gram negative. **Nonmotile** or **motile** by peritrichous flagella. **Strictly aerobic. Optimal temperature, 35–37°C.** Colonies on Bordet–Gengou medium are smooth, convex, pearly, glistening, nearly transparent, and surrounded by a zone of hemolysis without definite periphery. **Respiratory metabolism.** Chemoorganotrophic. **Require nicotinamide, organic sulfur** (e.g., cysteine), and **organic nitrogen** (amino acids). **Utilize oxidatively** glutamic acid, proline, alanine, aspartic acid, and serine, with production of ammonia and CO_2. Litmus milk is made alkaline. Mammalian and avian parasite and pathogen. Most species localize and multiply among the epithelial cilia of the respiratory tract.

The mol% G + C of the DNA is: 66–70.

Type species: **Bordetella pertussis** (Bergey, Harrison, Breed, Hammer and Huntoon 1923a) Moreno-López 1952, 178 (Microbe de coqueluche Bordet and Gengou 1906, 731; *Haemophilus pertussis* Bergey, Harrison, Breed, Hammer and Huntoon 1923a, 269.)

FURTHER DESCRIPTIVE INFORMATION

Phylogeny and taxonomy The genus *Bordetella*, along with the closely related genus *Alcaligenes*, constitute the family *Alcaligenaceae* of the *Betaproteobacteria*. The close association of these genera has been demonstrated by phenotypic analysis, which identified *Alcaligenes* as the nearest neighbor to *Bordetella* and most closely associated strains of this genus with *B. bronchiseptica* (Johnson and Sneath, 1973). DNA–rRNA hybridization and G + C content confirm this association, together with their mutual divergence from other taxa (De Ley et al., 1986).

Historically, it has been difficult to differentiate between *Bordetella* and *Alcaligenes* based on phenotype because of the low reactivity and common responses in many tests. Formerly, *Bordetella* spp. were regarded as pathogens of the upper respiratory tract of humans and animals and *Alcaligenes* spp. as aquatic and terrestrial saprophytes. This distinction, based on ecological niche, has been confounded by recent revelations in phylogeny, reclassification of some *Alcaligenes* spp., and the designation of novel *Bordetella* spp. that are not etiologic agents of respiratory diseases.

The genus *Bordetella* contains seven species: *B. pertussis, B. parapertussis, B. bronchiseptica, B. avium, B. hinzii, B. holmesii,* and *B. trematum.* Various methods have been used to derive phylogeny within the genus including analyses of phenotypic characteristics, DNA base composition, nucleic acid hybridization, multilocus enzyme electrophoresis (MEE), gene-sequence analyses, and the distribution and copy numbers of insertion sequences (Johnson and Sneath, 1973; Kloos et al., 1979; Kiredjian et al., 1981; Kersters et al., 1984; De Ley et al., 1986; van der Zee et al., 1997). In 1973, Johnson and Sneath described a numerical taxonomic study of *B. pertussis, B. parapertussis,* and *B. bronchiseptica* based on 134 characteristics. They showed that the test strains aligned into three highly similar clusters corresponding to the respective species. Differentiation based on whole-cell protein and fatty acid analysis also supported the species status of these taxa (Vancanneyt et al., 1995). DNA hybridization results subsequently yielded similar clustering, but the tested isolates were sufficiently related

as to constitute a single genomospecies (Kloos et al., 1979; Weyant et al., 1995a). Evaluations of the DNA base composition, DNA–rRNA hybridization results (De Ley et al., 1986), sequence similarity among the 23S rRNA genes (Muller and Hildebrandt, 1993), and genetic relatedness measured by multilocus enzyme electrophoresis (Musser et al., 1986) also supported the single-species concept. Conversely, lipopolysaccharide expression is nomenspecies-specific (van den Akker, 1998), and macro-restriction profiling of genomic DNA with a rarely cutting endonuclease demonstrated heterogeneity among the three species *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* (Khattak and Mathews, 1993). Although strains were more highly related within a given species, sufficient variability to discriminate between epidemiologically unrelated *B. pertussis* isolates has been documented.

Gene sequence analysis (Marchitto et al., 1987; Muller and Hildebrandt, 1993) and the data supporting the single-species concept suggest a relatively recent evolutionary origin and a common ancestor for *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* (Mooi et al., 1987; van der Zee, 1997). However, further elucidating the relationships within the genus was contentious until recently. *B. pertussis* and *B. parapertussis* were most closely related by the phenotypic characteristics evaluated (Johnson and Sneath, 1973). Sequence variations among pertussis toxin operons provided evidence for the evolution of *B. parapertussis* and then *B. bronchiseptica* from a common ancestor through *B. pertussis* (Arico and Rappuoli, 1987). DNA–rRNA hybridization studies separated *B. pertussis* and *B. bronchiseptica* with *B. parapertussis* between and overlapping them (De Ley et al., 1986). DNA hybridization (Kloos et al., 1979), multilocus enzyme electrophoresis (MEE) (Musser et al., 1986), and susceptibility to lytic bacteriophage (Rauch and Pickett, 1961) suggested that *B. parapertussis* is more highly related to *B. bronchiseptica* than to *B. pertussis*. The restricted diversity exhibited by MEE evaluations of *B. pertussis* and *B. parapertussis* isolates implies a shared ancestor (Musser et al., 1986). The comparatively greater heterogeneity found in *B. bronchiseptica* genetic structure, despite its general clonal nature, is consistent with an older lineage from which *B. pertussis* and *B. parapertussis* derived by host adaptation (Musser et al., 1986, 1987). Maximum parsimony evaluation of data from MEE and pertussis toxin operon sequences (Altschul, 1989) grouped all *B. pertussis* strains together with a unique common ancestor and designated *B. parapertussis* as a subtype of *B. bronchiseptica*.

MEE and DNA polymorphisms mediated by insertion sequence elements have recently yielded additional insights into phylogenetic relationships among *Bordetella* spp. (van der Zee et al., 1996, 1997). The distinction between sheep and human isolates of *B. parapertussis*, first based on differences in phenotype and electrophoretic types (Porter et al., 1994), and later reinforced by LPS expression (van den Akker, 1998), was confirmed by typing with insertion elements (van der Zee et al., 1996). In addition, this latter technique showed that transmission between the human and ovine populations was unlikely, and that the human strains were less divergent, suggesting that they evolved from *B. bronchiseptica* independently and more recently than sheep isolates.

Evaluation of 188 *Bordetella* isolates by MEE and the distribution of three insertion elements clarified the evolution and host adaptation in this genus (van der Zee et al., 1997). In general, host adaptation has restricted *B. pertussis* to humans and *B. parapertussis* to humans and sheep. Although some *B. bronchiseptica* clones tended to be more host- and geographically specific than others, it infects a much greater diversity of hosts, conforming with the greater genotypic diversity calculated for this species. Computer analyses of MEE along with distributions and sequences of insertion elements verified that *B. pertussis* and *B. parapertussis* descended from alternative clones of *B. bronchiseptica*, with *B. pertussis* infecting humans first. Indeed, *B. parapertussis* seems to have adapted to humans relatively recently from a *B. bronchiseptica* clade exclusive to pigs. In contrast, ovine strains of *B. parapertussis* most likely adapted to their host earlier.

Organisms belonging to four additional taxa are highly related to the historically recognized *Bordetella* spp. Among these were isolates classified as *Bordetella* or *Alcaligenes* causing an acute respiratory disease in birds that is symptomatically and pathologically analogous to pertussis in humans (Hinz et al., 1978; Simmons et al., 1981; Rimler and Semmons, 1983). The initial confusion over classifying such isolates was most likely due to their inactivity in conventional biochemical assays. Subsequently, several phenotypic approaches as well as DNA base composition and DNA–rRNA hybridization were used to assign such strains isolated from birds and causing coryza (rhinotracheitis) in turkey poults to a genotypically and phenotypically homogeneous fourth species, *B. avium* (Kersters et al., 1984). The DNA base composition of these strains was lower than those reported for the other *Bordetella* species and for the *Alcaligenes denitrificans–Achromobacter xylosoxidans* group (Kersters et al., 1984; De Ley et al., 1986). However, the 6.7 mol% G + C difference was reportedly insufficient to preclude *B. avium* from the genus, especially given the level of relatedness indicated by phenotypic properties, including isoprenoid quinone content (Fletcher et al., 1987), distinctive cellular fatty acid profiles (Moore et al., 1987), and DNA–rRNA hybridization (Kersters et al., 1984). Numerical analysis of phenotypic features related *B. avium* most closely to *B. parapertussis* (De Ley et al., 1986).

Bordetella holmesii was characterized in 1995 (Weyant et al., 1995a). Fifteen isolates representing this species were cultured from the blood of mostly young adults with underlying debilitations between 1983 and 1992. Initially referred to as CDC nonoxidizer group 2 (NO-2) based on common phenotypic characteristics, their cellular fatty acid profiles, 16S rRNA sequences, mol% G + C, and ubiquinone 8 content suggested close relatedness to the genus *Bordetella*. This was confirmed with DNA relatedness experiments that showed that these strains were sufficiently related as to constitute a single species (Wayne et al., 1987) and supported their placement within *Bordetella*. More recently, *B. holmesii*-like organisms were cultured from the sputum of a patient with acute pulmonary edema and bronchitis (Tang et al., 1998) and from 33 nasopharyngeal specimens collected from the nasopharynx of patients with cough illness as part of an active pertussis surveillance program (Yih et al., 1999). Additional studies are needed to define the role of *B. holmesii* in respiratory disease.

A sixth species is composed of isolates sharing similar features and resembling *B. avium* on biochemical assays that were cultured from the respiratory tracts of poultry and two human clinical specimens (Vandamme et al., 1995c). Although initially isolated in diverse geographical areas from turkeys and chickens with respiratory illness, there is insufficient evidence to attribute an etiologic role for these strains (Berkhoff and Riddle, 1984). Conversely, the human isolates from blood and sputum specimens were associated with bacteremia in an immunocompromised patient (Cookson et al., 1994; Vandamme et al., 1995c) and pulmonary complications in a cystic fibrosis patient (Funke et al., 1996), respectively. A third human strain was cultured from

human sputum, but no clinical significance could be assigned due to lack of clinical data (Kiredjian et al., 1981). Twelve isolates from poultry and two from humans were evaluated by nucleic acid composition and hybridizations, and their phenotypic characteristics, including protein and fatty acid analysis. These results revealed that these strains are a new species, *Bordetella hinzii* (Vandamme et al., 1995c).

Previous efforts to characterize atypical members of the *Bordetella–Alcaligenes* group led to the evaluation of a collection of 10 isolates recovered from wounds and chronic otitis media (Dorittke et al., 1995; Vandamme et al., 1996a). Comparison of protein electrophoretic patterns of these isolates disclosed highly similar patterns that were distinguishable from the protein profiles of representative strains of the *Alcaligenaceae*. DNA base composition and hybridization analyses confirmed the homogeneity of this group. DNA–rRNA hybridization results placed these strains within the *Alcaligenaceae* and aligned a representative strain more closely to the *Bordetella* than the *Alcaligenes* type species. Consequently, the species has been named *Bordetella trematum* (Vandamme et al., 1996a).

Cellular morphology   *Bordetella* cells usually appear as minute coccobacilli 0.2–0.5 × 0.5–2.0 μm. Cells of degraded strains may appear slightly larger. *B. trematum* cells may be slightly larger (0.5–0.6 × 1.0–2.4 μm) than those of the other species. Capsules have been observed with *B. pertussis*, *B. bronchiseptica*, and *B. trematum* (Lawson, 1940; Nakase, 1957; Vandamme et al., 1996a). Peritrichous flagella are observed on cells of the motile species, *B. bronchiseptica*, *B. avium*, *B. trematum*, and *B. hinzii*. Labow and Mosley have measured the periodic structure of *B. bronchiseptica* flagella as 19.0 by 13.9 nm (Labaw and Mosley, 1955). Pili-like filaments have been observed on cells of *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. avium* (Morse and Morse, 1970; Jackwood and Saif, 1987). Similar investigations have not as yet been reported for the more recently described species.

Nutrition and growth conditions   Bordetellae are obligate aerobes with an optimal growth temperature range of 30–37°C. The metabolism of these organisms is based on the oxidation of amino acids. Carbohydrates are generally not utilized. Nicotinamide, organic nitrogen, and organic sulfur (cystine, cysteine, or glutathione) are required for growth (Weiss, 1992). With the exception of *B. pertussis*, growth can be obtained on a wide variety of culture media, including trypticase soy agar with 5% sheep blood, heart infusion agar with or without 5% rabbit blood, MacConkey agar, and peptone agar. Cultivation of *B. pertussis* is more difficult because of its susceptibility to various compounds (unsaturated fatty acids, colloidal sulfur, or sulfides) found in most growth media. Charcoal horse blood agar and Bordet–Gengou agar contain adsorbants for these inhibitors and allow for the growth of *B. pertussis* (Hoppe, 1988; Weyant et al., 1996). Charcoal horse blood agar has a longer shelf-life than Bordet–Gengou agar and is superior in its ability to support *B. pertussis* growth (Hoppe, 1988). Two chemically defined media, Stainer-Scholte broth and cyclodextrin solid medium (Aoyama et al., 1986), have also been developed to support the growth of *B. pertussis*.

Colonial morphology   The time of development and the size of colonies differ among the species. *B. pertussis* is the most fastidious, with 3–6 d required for the development of pinpoint colonies on Bordet–Gengou medium. Colonies have a characteristic narrow zone of hemolysis and are usually convex and glistening, with an entire edge. Colony diameter rarely exceeds

3 mm. *B. parapertussis* and *B. holmesii* produce slightly larger convex, semi-opaque colonies at 2–3 d of incubation. Diffuse zones of browning may be observed around *B. parapertussis* and *B. holmesii* colonies, especially in media supplemented with tyrosine. Strains of the other *Bordetella* species usually produce colonies within 2 d of incubation. *B. bronchiseptica* colonies on MacConkey agar are reddish and surrounded by a small red zone with amber discoloration of the underlying medium. *B. trematum* strains produce convex, circular, and grayish cream white colonies with entire edges on blood agar (Vandamme et al., 1996a). Multiple colony types each have been described for *B. avium* and *B. hinzii*. For *B. avium*, type I colonies are small pearl-like with entire edges and glistening surfaces and <1 mm in diameter after 24 h of incubation. Type II colonies are larger, circular, and convex with entire edges and smooth surfaces. Colony types are usually strain-specific and stable (Kersters et al., 1984). A third colony type, characterized by a crenated edge and a flat surface with a wrinkled or ground-glass appearance, has also been described (Jackwood et al., 1991). Some strains of *B. hinzii* produce round, raised, glistening, grayish colonies about 2 mm in diameter at 48 h of incubation. Other strains produce flat, dry, crinkled colonies with diameters up to 5 mm at 48 h of incubation (Vandamme et al., 1995c).

Pathogenicity   *Bordetella* species are responsible for respiratory tract infections in humans and various other warm-blooded animals. These organisms have a significant public health and economic impact. *B. pertussis* causes pertussis (whooping cough) in humans. This disease is characterized by violent paroxysms of coughing followed by a loud inspiratory "whoop" (von Lichtenberg, 1984). The incubation period is between 7 and 10 d. The disease is highly communicable, with person-to-person transmission occurring via aerosolized droplet nuclei. Pertussis infections result in over 350,000 deaths annually (Cherry, 1996; Hoppe, 1999).

Current pertussis-containing vaccines are effective in preventing or attenuating severe pertussis after three or more doses. Because vaccine-induced immunity wanes with time and vaccination does not protect from *B. pertussis* infection, vaccinees of any age may contribute to the reservoir of endemic pertussis. Booster immunizations may be licensed for adolescents and adults in the near future and have the potential to decrease pertussis morbidity in these populations. This strategy may also reduce transmission to young infants, reducing morbidity and mortality among this highly susceptible group (Guris et al., 1999; Centers for Disease Control and Prevention, 2001).

B. parapertussis causes a milder pertussis-like disease in humans and also causes chronic progressive pneumonia in sheep (Martin, 1996; Hoppe, 1999). Studies in mice and specific pathogen-free lambs indicate that prior inoculation with *B. parapertussis* predisposes these animals to pneumonia caused by *Mannheimia* species (previously *Pasteurella haemolytica* complex) (Porter et al., 1995a, c). Pulsed-field gel electrophoresis of cellular DNA, cellular fatty acid profiles, and LPS characterization with monoclonal antibodies have been used to differentiate human from ovine isolates of *B. parapertussis* (Porter et al., 1995b, 1996).

Bordetella avium and *Bordetella bronchiseptica* are predominately animal pathogens. *B. avium* causes coryza or rhinotracheitis in birds, particularly turkeys (Arp and Cheville, 1984; Skeeles and Arp, 1997). This disease is of significant economic concern to worldwide agriculture (Blackall and Doheny, 1987; Skeeles and Arp, 1997). *B. bronchiseptica* causes respiratory tract infections in various animals, including swine, dogs, guinea pigs, rabbits, cats,

and horses (Roop, 1984). In swine, two forms of infection are recognized: atrophic rhinitis and bronchopneumonia in piglets. Atrophic rhinitis is a disease characterized by an initial episode of acute rhinitis, which is followed by chronic atrophy of the turbinate bones and deformity of the face. More severe cases of this disease are associated with dual infection by *B. bronchiseptica* and *Pasteurella multocida* (Rutter, 1985). Dogs (kennel cough) and guinea pigs in group housing are susceptible to outbreaks of respiratory infections caused by *B. bronchiseptica* (Bemis et al., 1977; Goodnow, 1980). *B. bronchiseptica* has also been isolated as a commensal organism in the upper respiratory tract of humans and has been implicated in infections of severely immunocompromised individuals (Woolfrey and Moody, 1991).

An extensive body of literature has been written to describe pathogenic mechanisms associated with *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. avium* (Wardlaw and Parton, 1988; Weiss, 1992; Parton, 1996). These species show a tropism for ciliated respiratory epithelial cells. Once attachment is achieved, various other virulence factors are produced that mediate damage and loss of ciliated cells and, in advanced cases, systemic pathology (Table BXII.β.32). Pili or fimbriae play a significant role in the early stages of pathogenesis. The major fimbrial subunits, agglutinogen 2/fimbria 2 and fimbria 3, mediate binding to host tissues (Miller et al., 1943; Sako, 1947; Preston, 1963; Preston and Stanbridge, 1972; Stanbridge and Preston, 1974; Preston et al., 1982, 1990; Mooi et al., 1992; Willems et al., 1992b; Hewlett, 1997; Preston and Matthews, 1998). Pertactin is a nonfimbrial surface protein associated with attachment (Charles et al., 1989, 1994; Hewlett, 1997). Nonsurface factors involved in the attachment process include tracheal colonization factor, serum resistance factor, and filamentous hemagglutinin (Van't Wout et al., 1992; Fernandez and Weiss, 1994; Finn and Stevens, 1995; Hewlett, 1997; Liu et al., 1997; Cotter et al., 1998).

Pertussis toxin is a complex protein that has attachment properties as well as other virulence functions (Katada and Ui, 1982; Pittman, 1984a; Weiss and Hewlett, 1986; Relman et al., 1990b; Sato and Sato, 1990; Granstrom et al., 1991; Van't Wout et al., 1992; Sandros and Tuomanen, 1993; Trollfors et al., 1995; Hewlett, 1997; Ui, 1998; Williamson and Matthews, 1999). The toxin contains two functional subunits: the B subunit facilitates binding to host cells and introduction of the enzymatically active A subunit into the host cell cytoplasm. The A subunit then acts as an ADP-ribosyltransferase that modifies and inactivates host cell G proteins. Other postattachment virulence factors include dermonecrotic toxin, which is a potent vasoconstrictor associated with ischaemia and skin necrosis in experimental animals (Livey and Wardlaw, 1984); tracheal cytotoxin, which inhibits DNA synthesis in ciliated epithelial cells (Goldman et al., 1982; Luker et al., 1993); adenylate cyclase toxin/hemolysin, which produces unregulated adenylate cyclase activity in host cells (Wolff et al., 1980; Hanski and Farfel, 1985; Friedman et al., 1987; Glaser et al., 1988; Gueirard et al., 1998; Harvill et al., 1999); and lipopolysaccharide, which produces endotoxin-like effects (Allen et al., 1998).

The expression of virulence in *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. avium* is an elegantly regulated phenomenon. In the premolecular era it was observed that *B. pertussis* isolates underwent a phase variation from a virulent (phase I) to avirulent (phase IV) phenotype in guinea pigs with passage *in vitro* (Leslie and Gardner, 1931; Pittman, 1984a). This reduction in virulence was accompanied by changes in colonial mor-

phology and serologic activity. Phase variation occurs with a frequency of 1 per 10^3–10^6 organisms and is generally irreversible *in vitro*. The molecular basis for phase variation was described by Weiss et al., who demonstrated by transposon insertion experiments that pertussis toxin, adenylate cyclase toxin/hemolysis, filamentous hemagglutinin, and dermatonecrotic toxin were all regulated by a single regulatory locus, designated *bvg* (Weiss et al., 1983; Weiss and Falkow, 1984). Mutations or deletions affecting the *bvg* locus result in phase variation. The expression of virulence factors is also affected by growth conditions, including temperature and salt concentration (Lacy, 1960). Unlike phase variation, this phenomenon, designated antigenic modulation, is freely reversible.

The pathogenic mechanisms and clinical significance of the more recently described *Bordetella* species have not yet been worked out. *B. hinzii* has been isolated from human and avian sources. Studies by Blackall and Doheny (1987) and Jackwood et al. (1985) suggest that this species is not a significant avian pathogen. In humans, *B. hinzii* has been isolated from the blood of a human immunodeficiency virus-infected patient and the sputa of two patients, one of whom had cystic fibrosis (Cookson et al., 1994; Vandamme et al., 1995c; Funke et al., 1996). *Bordetella holmesii* has been isolated from human blood cultures and from nasopharyngeal specimens of patients suspected of having pertussis (Lindquist et al., 1995; Weyant et al., 1995a; Mazengia et al., 2000). Some of the patients with positive blood cultures had been previously splenectomized (Lindquist et al., 1995; Weyant et al., 1995a). *Bordetella trematum* has been isolated from human wound and otitis media specimens (Vandamme et al., 1996a).

Ecology *Bordetella* species are isolated from warm-blooded animals, including humans. Until recently, the habitat of this genus was thought to be limited to the respiratory tract of the host. However, *B. holmesii* and *B. hinzii* have been isolated from human blood, and *B. trematum* has been isolated from various human wound cultures (Vandamme et al., 1995c, 1996a; Weyant et al., 1995a). The phenotypic similarity of *B. bronchiseptica*, *B. trematum*, and *B. hinzii* to the free-living *Alcaligenes* species suggests the potential for their isolation from the natural environment, although this has yet to be documented.

Isolation and direct detection procedures Bordetellae are commonly isolated from human specimens, and suspected cases of pertussis should have a nasopharyngeal aspirate or swab obtained from the posterior nasopharynx for culture for *B. pertussis* and *B. parapertussis*. Nasopharyngeal aspirates have similar or higher rates of recovery for *B. pertussis* than nasopharyngeal swabs, but recovery from throat and anterior nasal swabs is unacceptably low (Hoppe and Weiss, 1987; Halperin et al., 1989; Hallander et al., 1993; Bejuk et al., 1995). An enriched agar medium optimizes recovery of *B. pertussis* on primary culture. Modified B-G medium (Kendrick and Eldering, 1969) and charcoal agar with horse blood (Regan and Lowe, 1977) are most commonly used for primary isolation: the former allows for detecting hemolytic activity of *Bordetella* spp., the latter is reportedly more sensitive (Muller et al., 1997a). Supplemental antibiotics (penicillin at 0.25–0.5 U/ml for B–G and cephalexin at 40 µg/ml for charcoal-horse blood agar) are effective in inhibiting the growth of some normal nasopharyngeal flora. Plates incubated at 36° ± 1°C under ambient air and high humidity are examined daily until positive or for 7 d. Other primary isolation media (Lautrop, 1960; Imaizumi et al., 1983) have been proposed, but

TABLE BXII.β.32. Virulence factors of *Bordetella pertussis* and other *Bordetella* species

Virulence factors[a]	Molecular mass (kDA)	Mechanism of action	Stage of infection	Protective immunity	Location and other features	*bvg* regulated	Expression in other *Bordetella* sp.[b]
Agglutinogen2/fimbria 2	22	Fim2 binds heparin; fimD binds heparin and integrin VLA-5	A	+	Located on fimbriae (major subunit); confers protective immunity against serotypes 1 and 2	−	Bp, Bb, Baa
Agglutinin 3			A	+	Either a somatic or fimbrial antigen; confers protective immunity against serotypes 1 and 3	−	Bp, Bb, Baa
Fimbria 3	21.5	FimD binds to heparin and integrin VLA-5	A	+	Major fimbrial subunit	+	Bp, Bb, Baa
P.69 Pertactin	69	RGD motif, probably binds CR3	A	+	Somatic antigen	+	Bp, Bb
Pertussis Toxin	Subunits of 26, 22, 22, 12, 12, 11	Binds ciliated epithelium and macrophages; mimics selectins and upregulates macrophage CR3 for FHA binding; ADP ribosylation of cellular G proteins	A,D,L,S	+	Synergistic adhesin with FHA	+	−
Filamentous hemagglutinin	220	Binds both the bacterium and macrophage CR3 to facilitate phagocytosis	A	−	Secreted; synergistic adhesin with PT	+	Bp, Bb
Adenylate cyclase/hemolysin	45	ATP hydrolysis with raised intracellular cAMP in macrophages and lymphocytes; induces apoptosis	D,L,S	−	Secreted by type I pathway requiring CyaB, D and E proteins; activated by eucaryotic calmodulin	+	Bp, Bb
Tracheal cytotoxin	921	DNA inhibition in ciliated epithelium	D,L	−	A muramyl peptide, derived from bacterial peptoglycan	−	Bp, Bb, ba
Dermonecrotic toxin	102 with subunits of 30, 24	Inhibition of Na^+-K^+ ATPase; vasoconstriction	L,S		Localized to the bacterial cytoplasm; part of molecule probably exposed at cell surface		Bp, Bb, Ba
Lipopolysaccharide		Endotoxin-like effects; pyrogenic, sensitization to histamine	L.S		Two lipids, A and X; two different oligosaccharides, I and II		Bp, Bb, Ba
Tracheal colonization factor	64	Tcf binding, probably mediated by RGD motif	A		Secreted by type IV pathway	+	−
Serum resistance factor	BrkA: 103; BRKB: 32		D		Secreted by type IV pathway		−

[a]Fim2, fimbria 2; Fim3, fimbria 3; FHA, filamentous hemagglutination; PT, pertussis toxin; tcf, tracheal colonization factor; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; VLA-5, very-late antigen 5; CR3, complement receptor 3; ADP, adenosine diphosphate; cAMP, cyclic adenosine monophosphate; brk, *Bordetella* resistance to killing; RGD, Arg-Gly-Asp sequence; A, attachment; D, evasion of host defense; L, local effects; S, systemic effects.

[b]Bp, *B. parapertussis*; Bb, *B. bronchiseptica*; Ba, *B. avium*; fimbriae are produced by *B. parapertussis*, *B. bronchiseptica*, and *B. avium*, but are antigenically distant from *B. pertussis* fimbriae.

are not widely used. If direct inoculation of a selective medium is not possible, swab specimens can be placed in Regan–Lowe transport medium (one-half strength charcoal agar supplemented with horse blood and cephalexin) (Regan and Lowe, 1977). Some investigators recommend incubating the specimen (swab in the transport medium) before transport to the laboratory because incubating the specimen before transport reportedly allows for growth of *B. pertussis* (Strebel et al., 1993). Others suggest that this practice can decrease the yield of *Bordetella* spp. due to overgrowth by normal flora and recommend storage and transport at 4°C (Morrill et al., 1988). Culture of *B. pertussis* and *B. parapertussis* is optimal at 35–36°C, aerobically

(without CO_2), and with sufficient humidity to avoid desiccation. Cultures can be incubated for up to 7 d; *B. pertussis* rarely is visible before 3–4 d, but *B. parapertussis* is commonly discernible at least 24 h earlier.

Numerous studies have demonstrated the potential for polymerase chain reaction (PCR) assays to detect *Bordetella* cells with greater sensitivity and more rapidly than by culture. However, no specific technique for PCR is universally accepted or validated among laboratories. In addition, there is no quality assurance program for PCR. The use of PCR without culture negatively impacts the monitoring of cases of disease, recruitment of isolates for epidemiologic studies, and surveillance for antibiotic resis-

tance. For these reasons, PCR currently is best used as a presumptive assay in conjunction with culture under the conditions described in Meade and Bollen (1994).

Commercially available direct fluorescence assay (DFA) tests have been developed to detect *B. pertussis* in clinical specimens (Preston, 1970; Gilligan and Fisher, 1984; Ewanowich et al., 1993). Although these tests have been widely used to screen patients for *B. pertussis* infection, problems with specificity have been noted. Cross-reactions with normal nasopharyngeal flora accounted for false-positive results in up to 85% of tests (Ewanowich et al., 1993) and led to substantial unnecessary public health intervention. For the best results, DFA tests require care in all technical aspects and experienced personnel for their interpretation. If used, they should always be accompanied by a specimen for culture. Health care and public health workers can weigh the benefit of a presumptive diagnosis with the disadvantage of a high percentage of false-positive results. A mouse monoclonal antibody (BL-5)-based DFA test recently become available (Accu-MabTM, Biotex Laboratories Inc., Edmonton, Canada). Initial evaluation demonstrated 65% sensitivity and 99% specificity when compared with culture (McNicol et al., 1995). Further testing is necessary to confirm these results. The technical requirements and the careful standardization required for previous commercially available DFA tests also apply to this new monoclonal DFA; patients should also be cultured for isolation of *B. pertussis*.

Detection of antibodies to *B. pertussis* in humans Several strategies have been used to measure the immune responses to *B. pertussis* in patients and in vaccinees, including serum antibodies that agglutinate *B. pertussis* cells and a cytopathogenic assay for antibodies that neutralize pertussis toxin (PT). The predictive values of these assays for infection or protection are unacceptably low. Recent reports show promise in defining the role of serum bactericidal antibodies in pertussis immunity, but additional studies are needed. Enzyme-linked immunosorbent assays (ELISA) have provided the most sensitive and specific results, but the materials and methods required for them are not uniform. These tests also fail to relate a specific antibody to a specific protective function. None of the techniques has been validated between laboratories or been approved for diagnostic use in the United States. Currently, the most generally accepted serologic criterion for diagnosis of pertussis is the use of ELISA to demonstrate a significant increase in serum antibody concentrations against PT between acute and convalescent specimens. Anti-PT concentrations in convalescent sera may have diagnostic relevance in populations with known age-related prevalence of these antibodies, or in investigations where the prevalence among controls matched to cases by age and vaccination status can be determined. For individual patients, serology generally is regarded as an adjunct to culture rather than a method for primary diagnosis.

Antibiotic susceptibility Erythromycin is the antibiotic treatment of choice for *B. pertussis* infections (Hoppe et al., 1992). However, in recent years, erythromycin-resistant strains have been recovered (Lewis et al., 1995; Korgenski and Daly, 1997). The prevalence of these strains is not known but available evidence suggests that it is low. One of the erythromycin-resistant isolates was susceptible to trimethoprim-sulfamethoxazole (TMP-SMX), but cross-resistance to clarithromycin and clindamycin was demonstrated in the other. Susceptibility testing to a macrolide and to TMP-SMX can be considered for strains isolated from patients receiving at least 7 d of prophylaxis, or for purposes of

surveillance, but is not necessary for all patients. Disk-diffusion or E-Test (Korgenski and Daly, 1997) on charcoal agar containing horse blood have typically been used to screen for antibiotic susceptibilities of *B. pertussis*. There is less experience determining the minimum inhibitory concentrations (MIC) by agar dilution. Hoppe and Paulus suggest using Mueller-Hinton broth and agar with defibrinated horse blood and high inoculums of the test bacteria (Hoppe and Paulus, 1998). Erythromycin, TMP-SMX, and fluoroquinolones have high *in vitro* activities against *B. parapertussis* (Kurzynski et al., 1988; Hoppe and Simon, 1990; Hoppe and Tschirner, 1997).

In vitro antibiotic susceptibility studies with *B. bronchiseptica* indicate a resistance pattern similar to that observed with other nonfermentative Gram-negative bacilli (Kurzynski et al., 1988; Mortensen et al., 1989; Woolfrey and Moody, 1991). Strains of this species are usually sensitive to aminoglycosides, antipseudomonal penicillins, such as azlocillin, mezlocillin, and piperacillin, and broad-spectrum cephalothins, such as cefoperazone. High levels of resistance are usually seen with erythromycin and first- and second-generation penicillins and cephalosporins. Resistance to TMP-SMX and tetracyclines is variable. *In vitro* studies with *B. avium* show an overall lower level of antimicrobial resistance than with *B. bronchiseptica* (Mortensen et al., 1989). Strains of this species are usually sensitive to aminoglycosides, ampicillin, antipseudomonal penicillins, and tetracycline. Sensitivity to first- and second-generation cephalosporins is variable, and most strains are resistant to erythromycin and TMP-SMX (Mortensen et al., 1989; Blackall et al., 1995). *B. hinzii* has been reported to be susceptible to aminoglycosides and TMP-SMX, but resistant to ampicillin, erythromycin, and tetracycline (Blackall et al., 1995; Funke et al., 1996). Susceptibility studies with *B. holmesii* and *B. trematum* suffer from a lack of confirmed strains. Lindquist et al. (1995) described a single *B. holmesii* strain with disk diffusion results suggesting susceptibility to a wide range of agents, including aminoglycosides, TMP-SMX, tetracycline, ciprofloxacin, antipseudomonal penicillins, and ceftazidime.

MAINTENANCE PROCEDURES

For temporary preservation, *B. bronchiseptica*, *B. avium*, *B. hinzii*, *B. holmesii*, and *B. trematum* may be stored as stabs in motility medium containing 0.4% agar (Weyant et al., 1996). Screw-capped 15 × 125-mm tubes containing 8 ml of medium are inoculated with fresh agar-grown cultures by stabbing with a sterile needle or loop to approximately 2 cm below the agar surface. The tubes are then incubated at 35–37°C overnight with the caps loosened to allow for air exchange and then stored at room temperature with the caps tightened. *B. pertussis* and *B. parapertussis* may be grown and stored on B-G slants in closed containers at 4–6°C. Stored under these conditions, most strains will remain viable for 2–3 weeks. For permanent storage, fresh agar-grown cultures may be suspended in sterile skim milk of 10% powdered milk and freeze-dried (Novotny and Brookes, 1975). Alternatively, strains may be preserved by suspending fresh agar-grown cultures in defibrinated rabbit blood or 0.05 M Tris-HCl buffer (pH 7.6) with 20% glycerol and freezing in liquid nitrogen (Novotny and Brookes, 1975; Weyant et al., 1996).

DIFFERENTIATION OF THE GENUS *BORDETELLA* FROM OTHER GENERA

Phenotypic characteristics useful in the differentiation of *Bordetella* species are given in Table BXII.β.33. Although *Bordetella* species are highly related at the phylogenetic level, they are phenotypically heterogeneous. *B. pertussis* fails to grow on most com-

monly used growth media due to inhibition by various medium constituents including unsaturated fatty acids and metal ions (Rowatt, 1957). This characteristic is not observed with the other *Bordetella* species, although *B. holmesii* and *B. parapertussis* do not grow as rapidly on most media as do *B. bronchiseptica*, *B. avium*, *B. hinzii*, and *B. trematum*. *B. holmesii* and *B. parapertussis* also produce a characteristic brown diffusible pigment in heart infusion broth with tyrosine and are oxidase-negative. Urease activity is useful in differentiating *B. holmesii* from *B. parapertussis*. Of the four more rapidly growing species, *B. trematum* is unique in producing a negative oxidase reaction. The citrate, nitrate, and urease tests are useful in differentiating between *B. bronchiseptica*, *B. avium*, and *B. hinzii*. A more complete phenotypic characterization of the species of *Bordetella* is given in Table BXII.β.34.

Tests useful in differentiating *Bordetella* from other phenotypically similar genera are also given in Table BXII.β.33. From a phylogenetic prospective, *Bordetella*, *Achromobacter*, and *Alcaligenes* are closely related. This relatedness is reflected in the high level of phenotypic similarity observed between *Alcaligenes* and *Achromobacter* species and the motile and more rapidly growing species of *Bordetella* (*B. bronchiseptica*, *B. avium*, *B. hinzii*, and *B. trematum*). Historically, the ability of *Bordetella* to colonize mammalian and avian respiratory tracts was useful in differentiating these genera; however, the more recently described species—*B. holmesii*, *B. hinzii*, and *B. trematum*—have been isolated from nonrespiratory

sources. *Brucella* species, *Haemophilus* species, and asaccharolytic *Acinetobacter* species share phenotypic similarities with *B. pertussis*, *B. parapertussis*, and *B. holmesii*, and also may be isolated from clinical specimens. The requirement for X and/or V factors differentiates *Haemophilus* from *Bordetella*. Growth on blood agar, brown diffusible pigment production, and acidification of carbohydrates are useful in differentiating *Brucella* from nonmotile *Bordetella* species. Cellular fatty acid profiles have also been used successfully to differentiate among these organisms (Moore et al., 1987; Cookson et al., 1994; Vandamme et al., 1995c, 1996a; Weyant et al., 1995a; Funke et al., 1996). The predominant cellular fatty acids associated with each of these taxa are given in Table BXII.β.33.

TAXONOMIC COMMENTS

Tang et al. (1998) have described a series of *Bordetella holmesii*-like strains associated with septicemia, endocarditis, and respiratory failure in humans. These isolates are phenotypically consistent with *B. holmesii* and have 99.8% 16S rRNA gene sequence similarity. Additional studies will be required to determine the taxonomic status of these strains.

ACKNOWLEDGMENTS

TABLE BXII.β.33. Differentiation of *Bordetella* species and other morphologically and phenotypically similar taxa[a]

Characteristic	*B. pertussis*	*B. avium*	*B. bronchiseptica*	*B. hinzii*	*B. holmesii*	*B. parapertussis*	*B. trematum*	*Achromobacter* sp.	*Alcaligenes faecalis*	Asaccharolytic *Acinetobacter* sp.	*Brucella* sp.	*Haemophilus* sp.
Motility	−	+	+	+	−	−	+	+	+	−	−	−
Growth on:												
Blood agar	−	+	+	+	+	+	+	+	+	+	+	V
MacConkey agar	−	+	+	+	+	+	+	+	+	V	V	V
Simmons citrate agar	−	−	+	+	−	−	−	+	+	V	−	−
Oxidase activity (Kovacs reagent)	+	+	+	+	−	−	−	+	+	−	+[b]	+
Acid production from D-glucose	−	−	−	−	−	−	−	V	−	−	V	+
Nitrate reduction	−	−	v	−	−	−	v	+	−	−	+	nd
Gas from nitrate	−	−	−	−	−	−	v	v	−	−	V	nd
Urease activity	−	−	+	−	−	+	−	−	−	V	+	V
Diffusible brown pigment[c]	−	−	−	−	+	+	−	−	−	V	−	−
Major cellular fatty acids:[d]												
$C_{14:0}$		+			+			+	+			+
$C_{16:0}$	+	+	+	+	+	+	+	+	+	+	+	+
$C_{16:1\,\omega7c}$	+		+					+	+	+		+
$C_{17:0\,cyclo}$		+	+	+	+	+	+	+	+			
$C_{18:0}$	+	+		+	+	+	+					
$C_{18:1\,\omega7c}$											+	
$C_{18:1\,\omega9c}$										+		
$C_{19:0\,cyclo\,\omega7c}$											+	
$C_{14:0\,3OH}$	+											

[a]Symbols: + , >89% strains positive; V, 11–89% strains positive; − , fewer than 11% strains positive; nd, not determined. Data from Vandamme et al. (1996a) and Weyant et al. (1996).

[b]A small number of oxidase-negative *Brucella abortus* and *Brucella suis* strains have been reported (Weyant et al., 1996).

[c]Demonstrated on heart infusion agar with tyrosine (Weyant et al., 1996).

[d]Present at greater than 5% of total cellular fatty acids; $C_{16:1\,\omega7c}$, palmitoleic; $C_{16:0}$, palmitic; $C_{14:0\,3OH}$, β-hydroxymyristic; $C_{18:0}$, stearic; $C_{17:0\,cyclo}$, $C_{17:0\,cyclo\,\omega7c}$; $C_{14:0}$, myristic; $C_{18:1\,\omega9c}$, oleic; $C_{19:0\,cyclo\,\omega7c}$, lactobacillic; $C_{18:1\,\omega7c}$, *cis*-vaccenic. Data from Weyant et al. (1996) and Vandamme et al. (1996a).

TABLE BXII.β.34. Characteristics of *Bordetella* species[a]

Characteristic	*B. pertussis*	*B. avium*	*B. bronchiseptica*	*B. hinzii*	*B. holmesii*	*B. parapertussis*	*B. trematum*
Aerobic growth at 25°C	−	+	+	+	−	−	+
Aerobic growth at 42°C	−	+	+	+	−	−	+
Growth on:							
MacConkey agar	−	+	+	+	−	+	+
MacConkey agar containing 320mg/l tellurite	nd	−	−	−	−	+	−
Simmons citrate agar	−	−	+	+	−	−	−
Salmonella-Shigella agar	nd	+	+	+	−	−	+
Cetrimide agar	nd	−	−	−	−	−	−
Growth in nutrient broth	nd	+	+	+	V	+	+
Growth in nutrient broth with 6% NaCl	nd	V	V	+	−	−	+
Motility	−	+	+	+	−	−	+
Diffusible brown pigment	nd	−	−	−	+	+	−
Pigment produced from tyrosine	nd	−	−	−	+	+	−
Oxidase activity:							
Kovacs reagent	+	+	+	+	−	−	−
Gaby-Hadley reagent	+	+	+	+	−	−	−
Nitrate reduction (classical method):							
Formation of nitrite	−	−	V	−	−	−	V
Denitrification	−	−	V	−	−	−	V
Nitrate reduction (API 20NE test):							
Formation of nitrite	−	−	+	−	−	−	V
Denitrification	−	−	−	−	−	−	−
Urease activity	−	−	+	−	−	+	−
Tetrazolium reduction	nd	+	+	+	−	+	+
Alkalinization of litmus milk	nd	−	+	−	−	−	−
Alkali production from:							
Acetamide	nd	+	−	+	−	−	V
Adipate	nd	+	+	+	−	−	+
Glycine	nd	−	+	+	−	−	−
Propionamide	nd	+	+	+	−	−	+
Valerate	nd	−	+	+	−	−	V
Malonamide	nd	−	+	+	−	−	+
Malonate	nd	−	+	+	−	−	+
Malonate (API ID 32E test)	−	−	−	−	−	−	−
Glucose fermentation	−	−	−	−	−	−	−
Assimilation of:							
D-Glucose	−	−	−	−	−	−	−
D-Xylose	−	−	−	−	−	−	−
D-Gluconate	−	−	−	−	−	−	−
D-Mannitol	−	−	−	−	−	−	−
Lactose	−	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−	−
Maltose	−	−	−	−	−	−	−
Caprate	−	−	V	+	−	−	−
Adipate	−	+	+	+	−	−	+
L-Malate	−	+	V	+	−	−	+
Phenylacetate	−	+	+	+	−	−	+
Esculin	−	−	−	+	−	−	+
5-Keto-gluconate	−	−	−	−	−	−	V
Browning on 2-keto-gluconate	−	−	−	+	+	−	−
Alkaline phosphatase activity	−	V	V	+	+	−	+
Lysine decarboxylase activity	−	−	−	−	−	−	−
Ester C₈ lipase activity	+	V	V	+	−	+	+
Lipase C₁₄ activity	−	−	−	−	−	−	V
Trypsin activity	−	−	−	−	−	−	−
Chymotrypsin activity	+	V	−	−	+	+	V
Naphthol-AS-B1-phosphohydrolase activity	+	−	−	+	+	−	+
Arylamidase activity with:							
Valine	−	V	−	+	−	−	+
Cystine	−	−	−	−	−	−	−
Arginine	+	V	+	+	+	−	V
Proline	nd	−	−	−	+	−	−
Leucyl glycine	+	+	+	+	+	−	+
Phenylalanine	+	+	−	−	−	−	V
Pyroglutamic acid	−	−	−	−	−	−	−
Tyrosine	+	+	+	+	−	−	V
Glutamyl glutamic acid	+	−	−	−	−	−	V
Serine	+	+	+	+	−	−	+
L-Aspartic acid	+	+	+	+	−	+	+

[a]Symbols: +, >89% strains positive; V, 11–89% strains positive; −, <11% strains positive; nd, not determined. Data from Vandamme et al. (1996a) and Weyant et al. (1996).

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List of species of the genus Bordetella

1. **Bordetella pertussis** (Bergey, Harrison, Breed, Hammer and Huntoon 1923a) Moreno-López 1952, 178[AL] (Microbe de coqueluche Bordet and Gengou 1906, 731; *Haemophilus pertussis* Bergey, Harrison, Breed, Hammer and Huntoon 1923a, 269.)

   per.tus'sis. L. pref. *per* very, severe; L. n. *tussis* cough; M.L. gen. n. *pertussis* of a severe cough, of whooping cough.

   Minute coccobacillus, 0.2–0.5 $\times$ 0.5–1.0 µm in length, encapsulated or surrounded by a slime sheath composed of extruded filaments or secreted blebs. Potato–glycerol–blood agar (Bordet–Gengou) has been preferred for primary isolation. Colonies are minute in size and are surrounded by a zone of hemolysis on media containing about 20% blood. Uniqueness in slow rate of growth and susceptibility to growth inhibitors are associated with the production of pertussis toxin. A defined broth medium may be used for mass growth. No pellicle is formed. Physiological and nutritional characteristics are presented in Tables BXII.β.33 and BXII.β.34. Parasitic, pathogenic, found only in the respiratory tract of humans, where it is the cause of pertussis (whooping cough).

   The mol% G + C of the DNA is: 66–70 (T_m).
   Type strain: 18-232 , ATCC 9797, DSM 5571.
   GenBank accession number (16S rRNA): U04950.

2. **Bordetella avium** Kersters, Hinz, Hertle, Segers, Lievens, Siegmann and De Ley 1984, 65[VP]

   a'vi.um. L. n. *avis* bird; L. gen. pl. n. *avium* of birds.

   The morphology is as described for the genus. Colonies appear earlier on Bordet–Gengou medium and are larger than those of *B. pertussis* or *B. parapertussis*. Grows on MacConkey agar. The physiological and nutritional characteristics are presented in Tables BXII.β.33 and BXII.β.34. Found in avian species and pathogenic for turkey poults.

   The mol% G + C of the DNA is: 61.6–62.6 (T_m).
   Type strain: Hinz 591-77, ATCC 35086, DSM 11332, LMG 1852.
   GenBank accession number (16S rRNA): U04947.

3. **Bordetella bronchiseptica** (Ferry 1912) Moreno-López 1952, 178[AL] (*Bacillus bronchicanis* Ferry 1911, 404; *Bacillus bronchisepticus* Ferry 1912, 377.)

   bron.chi.sep'ti.ca. Gr. n. *bronchus* the trachea; Gr. adj. *septicus* putrefactive, septic; M.L. fem. adj. *bronchiseptica* intended to mean with an infected bronchus.

   The morphology is as described for the genus. Colonies appear earlier on Bordet–Gengou medium and are larger than those of *B. pertussis* or *B. parapertussis*. Grows on MacConkey agar. A pellicle forms on liquid media. The physiological and nutritional characteristics are presented in Tables BXII.β.33 and BXII.β.34. Parasitic, pathogenic, found in the respiratory tract of domestic and wild mammalian animals (dogs, swine, guinea pigs, rabbits, raccoons, etc.), causes kennel cough in dogs.

   The mol% G + C of the DNA is: 67–69 (T_m).
   Type strain: 71, ATCC 19395, NCTC 452.
   GenBank accession number (16S rRNA): U04948.

4. **Bordetella hinzii** Vandamme, Hommez, Vancanneyt, Monsieurs, Hoste, Cookson, Wirsing von König, Kersters and Blackall 1995c, 43[VP]

   hin'zi.i. N.L. gen. n. *hinzii* of Hinz, named in honor of K.-H. Hinz.

   The cellular morphology is as described for the genus. Two distinct colony types occur. Some strains produce round, raised, glistening, grayish colonies about 2 mm in diameter following 48 h of incubation at 37°C in air containing 5% CO_2. Under the same conditions other strains produce flat, dry, crinkled colonies that are up to 5 mm in diameter. The physiological and nutritional characteristics are presented in Tables BXII.β.33 and BXII.β.34. Strains have been isolated from respiratory tracts of turkeys and chickens. Pathogenic in humans, with strains isolated in blood and sputum specimens. The type strain was isolated from a chicken trachea in Australia.

   The mol% G + C of the DNA is: 65–67 (T_m).
   Type strain: TC58, LMG 13501, ATCC 51783, CIP 104527, DSM 11333, LMG 13501.
   GenBank accession number (16S rRNA): AF177667.

5. **Bordetella holmesii** Weyant, Hollis, Weaver, Amin, Steigerwalt, O'Connor, Whitney, Daneshvar, Moss and Brenner 1995b, 619[VP] (Effective publication: Weyant, Hollis, Weaver, Amin, Steigerwalt, O'Connor, Whitney, Daneshvar, Moss and Brenner 1995a, 6.)

   holmes'i.i. N.L. gen. n. *holmesii* named in honor of B. Holmes.

   Cellular morphology is as described for the genus. Growth characteristics on blood agar are similar to those of *B. parapertussis*. Growth occurs on MacConkey agar at between 3 and 7 days of incubation at 35°C. The physiological and nutritional characteristics are presented in Tables BXII.β.33 and BXII.β.34. Strains have been isolated from blood and upper respiratory tract cultures of humans. The type strain was isolated from human blood.

   The mol% G + C of the DNA is: 61.5–62.3 (T_m).
   Type strain: ATCC 51541, CDC F5101.
   GenBank accession number (16S rRNA): U04820.

6. **Bordetella parapertussis** (Eldering and Kendrick 1938) Moreno-López 1952, 178[AL] (*Bacillus parapertussis* Eldering and Kendrick 1938, 571.)

   pa.ra.per.tus'sis. Gr. prep. *para* resembling; M.L. n. *pertussis* the specific epithet of *Bordetella pertussis*; M.L. adj. *parapertussis* resembling *Bordetella pertussis*.

   The morphology is similar to that of *B. pertussis*. Colonies are slightly larger and appear earlier on Bordet–Gengou medium than *B. pertussis*. Growth on peptone agar is accompanied by a brown discoloration of the medium that is

attributed to the action of tyrosinase on the tyrosine in the medium (Ensminger, 1953). A pellicle forms on liquid media. Growth is more rapid than with *B. pertussis*. The physiological and nutritional characteristics are presented in Tables BXII.β.33 and BXII.β.34. Parasitic, pathogenic, found in the respiratory tract of humans and sheep. Causes a milder pertussis-like syndrome in humans and chronic progressive pneumonia in ovines.

> *The mol% G + C of the DNA is*: 66–70 (T_m).
> *Type strain*: 522, ATCC 15311, NCTC 5952.
> *GenBank accession number (16S rRNA)*: U04949.

7. **Bordetella trematum** Vandamme, Heyndrickx, Vancanneyt, Hoste, De Vos, Falsen, Kersters and Hinz 1996a, 857[VP]

tre.ma′ tum. Gr. neut. n. *trema* referring to something pierced or penetrated, an aperture, or a gap; N.L. gen. pl. n. *trematum* pertaining to penetrated or open things, referring to the presence of these bacteria in wounds and other exposed parts of the human body.

Cellular morphology is as described for the genus. Strains do not require special growth factors and grow on conventional media. Colonies may be observed at 16–24 h of incubation at 37°C under aerobic conditions. The physiological and nutritional characteristics are presented in Tables BXII.β.33 and BXII.β.34. Strains have been isolated from wound and ear infections of humans.

> *The mol% G + C of the DNA is*: 64–65 (T_m).
> *Type strain*: CCUG 32381, DSM 11334, LMG 13506.
> *GenBank accession number (16S rRNA)*: AJ277798.

Genus IV. **Derxia** Jensen, Petersen, De and Bhattacharya 1960, 193[AL]

CHRISTINA KENNEDY

Derx′ i.a. M.L. fem. n. *Derxia* named after the Dutch microbiologist H.G. Derx (1894–1953).

Cells are rod-shaped with rounded ends, 1.0–1.2 µm × 3.0–6.0 µm, occurring singly or in short chains. **Cells are rather pleomorphic**, depending on age and the medium. In aging cultures cells often remain together forming long filaments of sometimes locally swollen or distorted cells. Some cells may assume very large lengths (up to 30 µm). **Young cells have a homogeneous cytoplasm; older cells show typical large refractile bodies—probably poly-β-hydroxybutyrate—throughout the whole cell**. Resting stages are not known. Gram negative. **Motile by a short polar flagellum**; motile cells are numerous in liquid glucose media containing combined nitrogen, but rare on nitrogen-deficient solid media. **Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. **N₂ is fixed under aerobic or microaerobic conditions**. Optimal temperature, 25–35°C; growth is slow at 15°C, feeble at 40°C; no growth at 50°C. **Growth occurs between pH 5.5 and ~9.0**; no growth at pH 4.4. **Broth cultures turn into a gelatinous mass**, but growth near the surface is more luxuriant and forms a thick, tough pellicle. **Colonies on agar media are at first slimy and semitransparent, later massive and opaque, highly raised with a wrinkled surface. Older colonies develop a dark mahogany-brown color. Catalase negative**. A wide range of sugars, alcohols, and organic acids are oxidized mostly to CO_2 and a small amount of acid, probably acetic, when growing in an alkaline medium. **Can grow as a facultative hydrogen autotroph. Found in tropical soils** (Asia, Africa, South America).

The mol% G + C of the DNA is: 69.2–72.6.

Type species: **Derxia gummosa** Jensen, Petersen, De and Bhattacharya 1960, 193.

FURTHER DESCRIPTIVE INFORMATION

The description, above and below, of the genus, as given by J.-H. Becking in the 1st edition of the *Manual* remains largely unchanged; information has been updated.*

Cell morphology The appearance of cells from young cultures is depicted in Fig. BXII.β.22. Older cells on sugar-rich media contain large refractile bodies. On glucose–peptone agar especially, very elongated cells are produced containing many refractile and misshapen bodies (Becking, 1984b). The refractile material is probably poly-β-hydroxybutyrate since it stains with Sudan III and Sudan black, but some vacuoles do not stain (Becking, 1984b). On nitrogen-free glucose agar older cells undergo shrinkage and are finally enclosed by a slime envelope (Fig. BXII.β.23).

Motile cells may become numerous in liquid glucose medium containing ammonia or glutamate as the nitrogen source. The cells usually have a single polar flagellum, but some may have a flagellum at each pole. According to Thompson and Skerman (1979), the single polar flagellum is less than a full wave and is less than 3 µm in length.
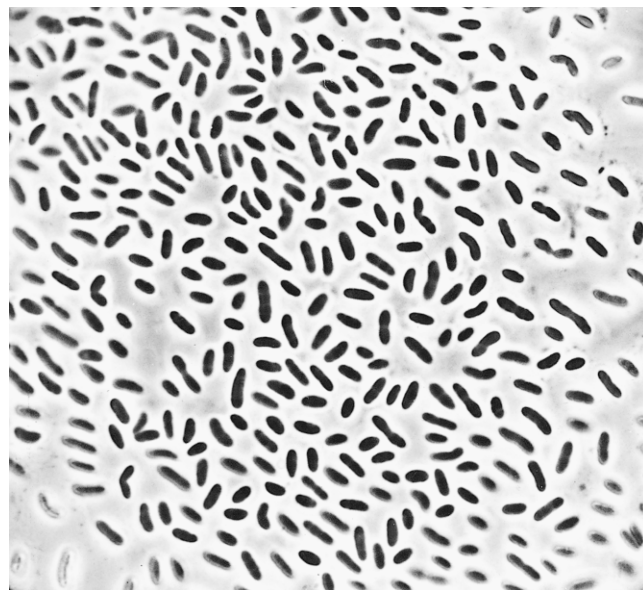

FIGURE BXII.β.22. Seven-day-old cells of *D. gummosa* on nitrogen-free agar containing 2% glucose. Phase-contrast microscopy (× 950).

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*Editorial Note: Derxia was placed in the *Beijerinckiaceae* in the first edition of the *Manual*; because the 16S rDNA sequence of *Derxia gummosa* had not been published. However, now because of the 16S rDNA placement, the genus *Derxia* has been moved to the family *Alcaligenaceae* and is no longer listed in *Beijerinckiaceae*.
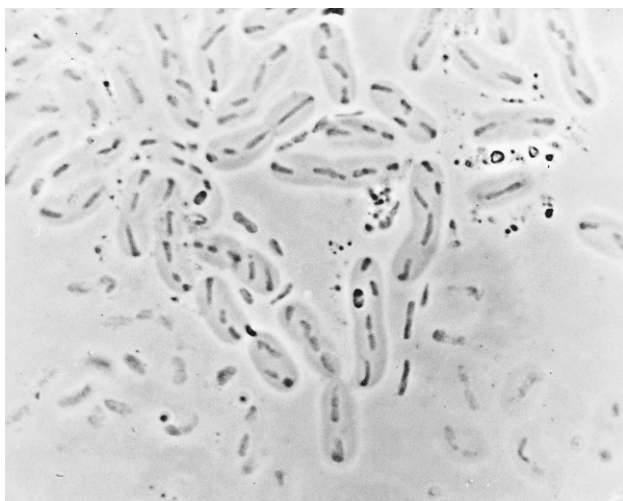
FIGURE BXII.β.23. Three-month-old cells of *D. gummosa* on nitrogen-free glucose agar. The cells show shrinkage and are enclosed by a thick slime envelope. Phase contrast microscopy (× 950).

Colonial and cultural characteristics Growth in liquid media usually starts as a ring at the glass-liquid interface and develops into a thick, wrinkled, tough pellicle. Shallow layers of medium change into a firm gelatinous mass after 2 weeks. The color gradually becomes a dark red-brown.

Growth on nitrogen-deficient agar media begins as thin, whitish or semitransparent scattered colonies. Later, more massive, highly raised or dome-shaped colonies emerge that rapidly assume a diameter of 1 cm or more (giant colonies) (Fig. BXII.β.24). These colonies are very like those of *Beijerinckia* spe-

FIGURE BXII.β.24. Colony type of *D. gummosa* on nitrogen-free glucose agar with calcium carbonate (× 1.0).

cies. For further comparisons of the differential characteristics and carbon compounds of *Beijerinckia* and *Derxia*, see Tables BXII.α.141 and BXII.α.142 in *Beijerinckia*. Colonies are at first whitish or dull yellow with a smooth surface, but the surface soon becomes coarse and wrinkled and the color deepens to a dark mahogany-brown. The slime of these colonies is very tenacious and gumlike, but in the other developmental stages it is more soft and smeary.

As noted by Jensen et al. (1960) in the original description of *D. gummosa*, aerobic growth on nitrogen-free, mineral glucose agar[1] consists of a mixture of a few "massive" colonies among many "thin," whitish colonies. It is the former that are associated with N_2 fixation; their slime affords some protection to the oxygen-sensitive nitrogenase system by decreasing the penetration of oxygen to the cells (Hill and Postgate, 1969). The occurrence of two kinds of colonies under aerobic conditions is probably due to the occurrence of local areas of decreased oxygen concentration on the plates. It is only in these areas that N_2 fixation and extensive cell multiplication can occur that leads to the formation of the "massive" colonies. Under microaerobic conditions (pO_2 <0.2 atm), all of the colonies on a plate develop into the "massive" type (Hill, 1971).

Metabolism and metabolic pathways As for *Beijerinckia* species, *Derxia* can utilize many sugars, organic acids, and alcohols for growth (see Table BXII.α.142 in Genus *Beijerinckia*). Growth on methane or methanol as sole carbon source has also been reported for *D. gummosa* (Sampaio et al., 1981).

The efficiency of N_2 fixation by *D. gummosa* varies between 9 and 25 mg N/g of glucose consumed, but in most strains it is distinctly lower than in *Azotobacter* or *Beijerinckia*. There is no requirement for amino acids, vitamins, or growth factors, but trace elements, particularly molybdenum, are required. Vanadium cannot replace the molybdenum for N_2 fixation, suggesting that the alternative V nitrogenase (nitrogenase-2) is not present (for further explanation see the genus *Azotobacter*). Nitrite but not nitrate inhibits nitrogenase activity as do ammonia and glutamine (Wang and Nicholas, 1986c).

Growth with combined nitrogen sources is much faster than with N_2 and is completely uniform, in contrast to the uneven growth on nitrogen-free agar. Colonies change from a pale yellow through rust-brown to almost black (darkest if nitrate is present) and sometimes a light brown, water-soluble pigment is produced. Growth with glutamic acid, ammonium acetate, alanine, sodium nitrate, and urea decreases from abundant to good in approximately the same sequence (Becking, 1984b). Aspartic acid, asparagine, and peptone give a much slower growth that is uneven and mostly confined to scattered colonies. Glycine seems to be toxic.

Although Thompson and Skerman (1979) reported four of six strains tested as able to utilize nitrate as N source, these strains gave negative results in tests of nitrite production from nitrate (see also Becking, 1984b). The reason for this discrepancy is not clear, but it is certain that some strains have nitrate reductase. The enzyme was purified from *D. gummosa* (unnamed strain supplied by Y.T. Tchan) and contained two dissimilar subunits, 80 and 88 kDa in size (Wang and Nicholas, 1986a). Induction of

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1. Nitrogen-free, glucose mineral medium (g/l distilled water): K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.25 (or 0.2); NaCl, 0.25; $FeSO_4 \cdot 7H_2O$, 0.1; $CaCl_2$ or $CaCO_3$, 0.1; $Na_2MoO_4 \cdot 2H_2O$, 0.005; and glucose, 10.0 (or 20.0); pH, 6.9. For a solid medium, add 15.0 g agar per liter.

nitrate reductase formation by nitrate was inhibited by ammonium or glutamine. Nitrite reductase was also purified (this enzyme also reduces hydroxylamine) (Wang and Nicholas, 1986d). Thus, at least some isolates of *D. gummosa* have both enzymes needed for conversion of nitrate to ammonia (assimilation).

As with most other *Proteobacteria*, *Derxia gummosa* assimilates ammonia via glutamine synthetase/glutamate synthase. Both enzymes were purified (Wang and Nicholas, 1985) and found to have physical and biochemical properties similar to these enzymes in other microorganisms, including adenylation of glutamine synthetase in response to ammonium.

D. gummosa grows autotrophically in an atmosphere containing $H_2/CO_2/O_2$, with either N_2 or NH_4^+ as the nitrogen source (Pedrosa et al., 1980). Indeed, it appears to grow nearly as well autotrophically as it does heterotrophically. Ribulose-1,5-bisphosphate activity, which mediates the CO_2 fixation, occurs in autotrophically grown cells but not in cells grown heterotrophically. Shankar et al. (1986) found that rapid O_2 depletion in a closed system caused variable, sometimes extremely poor, autotrophic growth; these authors developed a flow-through culturing technique that gave consistent results. Hydrogen recycling also takes place via an electron transport chain consisting of flavoprotein, ubiquinone, cytochrome *b*, and cytochrome *a* (Wang and Nicholas, 1986b).

Indole may or may not be produced from tryptophan. Starch is not hydrolyzed. *D. gummosa* antagonizes the growth of Gram-positive bacteria (Thompson and Skerman, 1979). Growth occurs on 1% peptone.

Ecology *Derxia* occurs mainly in tropical soils. It was isolated originally by Jensen et al. (1960) from a West Bengal soil having a pH of 6.5. It is widely distributed in Brazil (Campêlo and Döbereiner, 1970), Indonesia, China, and southern Africa (Becking, 1981). In Brazil, Campêlo and Döbereiner (1970) found it to be most frequent in flooded soils, less frequent in humid soils, and least frequent in dry soils. In comparing isolations made on a nitrogen-free, mineral starch agar, they found that *Derxia* could be more often isolated from root pieces of plants (mostly Gramineae) than from soil samples obtained from the same locality. In soil, the presence of the organisms seemed to be favored when the soil pH was between 5.1 and 5.5, although isolations were made from soils over a pH range of 4.5–6.5.

ENRICHMENT AND ISOLATION PROCEDURES

The sieved soil method may be used for isolation. Soil particles are regularly distributed over the surface of nitrogen-free mineral media with glucose[2] (Becking, 1984b). Mannitol and starch were previously suggested to be suitable C sources for isolation of *Derxia* (Jensen et al., 1960; Campêlo and Döbereiner, 1970), but Thompson and Skerman (1979) found all six strains tested unable to hydrolyze starch, and three of them were unable to utilize mannitol. Consequently, the use of mannitol or starch for isolation/enrichment of *Derxia* is not recommended. After growth on glucose N-free medium, one looks for the development of yellowish colonies around the soil particles. These colonies eventually become much larger and acquire a rust-brown color. Isolates should be further purified by repeated streaking.

Derxia strains are acid-tolerant and have been isolated from cultures designed to enrich for *Beijerinckia* strains by use of a liquid acidic nitrogen-free glucose medium[3] (Becking, 1984b). This medium is dispensed into Petri dishes so as to form a thin layer 2–3 mm deep.

MAINTENANCE PROCEDURES

The maintenance procedures are the same as those described for the genus *Beijerinckia*.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

The ability to grow autotrophically can be tested on a solid or liquid medium[4] in the presence or absence of $(NH_4)_2SO_4$ (1.0 g/l). Cultures are incubated at 30°C in sealed vessels under the following atmospheres: (a) O_2/N_2, 1:99, v/v; (b) $0_2/CO_2/N_2$, 1:5:94; (c) $O_2/H_2/N_2$, 1: 5:94; and (d) $O_2/H_2/CO_2/N_2$, 1:5:5:89. (Under atmospheres containing CO_2 the medium may also need to contain $NaHCO_3$ (1.0 g/l) to prevent acidification.) Significant growth should occur only when both H_2 and CO_2 are provided, even when N_2 is the sole nitrogen source. Under conditions of H_2/CO_2 dependent autotrophic growth under N_2-fixing or non-N_2-fixing conditions, the average doubling time of *D. gummosa* is 34.1 h (Pedrosa et al., 1980). A flow-through system for culturing *D. gummosa* autotrophically was also described (Shankar et al., 1986).

DIFFERENTIATION OF THE GENUS *DERXIA* FROM OTHER GENERA

Derxia can be distinguished from other genera of N_2-fixing bacteria by its very slimy (gummy) growth, both on agar plates and in liquid media, combined with appearance of the cells (Figs. BXII.β.22 and BXII.β.23) depending on age and type of medium (cells on peptone medium are very pleomorphic). *Azotobacter* and *Azomonas* species never produce such slimy colonies. Confusion with *Beijerinckia* is unlikely since *Beijerinckia* cells usually show very characteristic cells with two polar bodies containing PHB, whereas *Derxia* cells contain numerous PHB-containing bodies. Moreover, in contrast to *Beijerinckia* colonies, the colonies of *Derxia* acquire on aging a typical dark mahogany-brown color. In addition, *Derxia* is catalase negative, whereas *Beijerinckia* is catalase positive.

Distinction of *Derxia* and *Beijerinckia* species is also demonstrated by the differential ability to utilize compounds as carbon sources (see Table BXII.α.142 in the genus *Beijerinckia*). Most useful are possibly malonate, aspartate, glutamate, and ethylamine, carbon sources on which *D. gummosa* can grow but not species of *Beijerinckia* (Thompson and Skerman, 1979). In addition, resistance/sensitivity to antibiotics distinguish these genera. *D. gummosa* is more resistant to chlortetracycline and sulfanilamide than are *Beijerinckia* species (25 versus 1 µg/ml and

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2. Nitrogen-free, mineral glucose agar for sieved-soil method (g/l distilled water): glucose, 20.0; K_2HPO_4, 0.8; KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.5; $FeCl_3 \cdot 6H_2O$, 0.025 (or 0.05); $Na_2MoO_4 \cdot 2H_2O$, 0.005; $CaCl_2$, 0.05; and agar, 15.0. The pH is adjusted to 6.9.

3. Liquid acidic nitrogen-free glucose medium (g/l distilled water): glucose, 20.0; KH_2PO_4, 1.0; and $MgSO_4 \cdot 7H_2O$, 0.5. The pH is adjusted to 5.0.

4. Medium for testing autotrophy (per liter of distilled water): KH_2PO_4, 1.2 g; K_2HPO_4, 0.8 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; $FeSO_4 \cdot 7H_2O$, 0.002 g; trace element solution (see below), 2.0 ml; biotin, 10 µg; and agar, 15.0 g. Trace element solution (g/l): $Na_2MoO_4 \cdot 2H_2O$, 1.0; $MnSO_4 \cdot H_2O$, 1.75; H_3BO_3, 1.4; $CuSO_4 \cdot 5H_2O$, 0.04; and $ZnSO_4 \cdot 7H_2O$, 0.12. This medium was employed by Pedrosa et al. (1980) in their studies of *Derxia*. In those studies, a very low concentration of potassium malate (0.1 g/l) was sometimes added to the medium. It should be noted that most strains of *Derxia* (including the type strain) give only scant growth with malate. Thompson and Skerman (1979) reported no growth on DL-malate in all of the six strains that they tested.

125 versus 1–5 µg/ml, respectively). *Beijerinckia* species are more resistant to chloramphenicol and penicillin than is *D. gummosa* (125 versus 5 µg/ml and 25 versus 1 µg/ml, respectively) (Thompson and Skerman, 1979). *D. gummosa* can utilize glutamate as a nitrogen source, whereas species of *Beijerinckia* cannot. Unlike most other aerobic diazotrophic species, *Derxia* can grow as a facultative hydrogen autotroph.

TAXONOMIC COMMENTS

Derxia, like *Beijerinckia*, was previously classified in the family *Beijerinckiaceae* in the order *Rhizobiales*, class *Alphaproteobacteria*. This placement was based on phenotypic characterization, not 16S rDNA sequence. De Smedt et al. (1980), based on rRNA hybridization, placed *D. gummosa* in the third rRNA superfamily, which also contains *Pseudomonas solanacearum*, *Chromobacterium violaceum*, *Janthinobacterium lividum*, and *Alcaligenes faecalis*. Phenotypically, both *Derxia* and *Beijerinckia* species produce a viscous, tenacious slime and form colonies that are similar in several respects. They utilize similar carbon sources, and they have a molybdenum requirement for N_2 fixation that cannot be replaced by vanadium (Thompson and Skerman, 1979; Becking, 1984a, b) (see also Tables BXII.α.141 and BXII.α.142 in the genus *Beijerinckia*). In addition, both are isolated from similar habitats. It is likely that other species of *Derxia* besides *D. gummosa* exist, but none have been formally named. Roy and Sen (1962) described a new species of *Derxia* from a sample of jute from Uttar Pradesh, India. Wu and Chen (1991) proposed a new species, "*Derxia peritricha*", for an isolate that shared many cultural characteristics with *D. gummosa*, and DNA hybridization studies also indicated significant similarity.

FURTHER READING

Becking, J.H. 1992. The Genus *Derxia*. *In* Balows, Trüper, Dworkin and Schleifer (Editors), The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, Springer-Verlag, New York. pp. 2605–2611.

List of species of the genus Derxia

1. **Derxia gummosa** Jensen, Petersen, De and Bhattacharya 1960, 193[AL]

   gum.mo′sa. L. fem. adj. *gummosa* slime (gum) producing.

   The characteristics are as described for the genus. Originally isolated from a soil of West Bengal of pH 6.5, but later also from slightly acidic or neutral soils of South America (Brazil, Surinam), South Africa, and Java (Becking, 1984b).

   The mol% G + C of the DNA is: 70.4–71.7 (T_m) (De Ley and Park, 1966); 69.2–72.6 (T_m) (De Smedt et al., 1980).

   Type strain: ATCC 15994, DSM 723, NCIB 9064.

Genus V. **Oligella** Rossau, Kersters, Falsen, Jantzen, Segers, Union, Nehls and De Ley 1987, 205[VP]

KAREL KERSTERS AND MARC VANCANNEYT

O.li.gel′ la. Gr. adj. *oligos* little, scanty; M.L. dim. ending -*ella*; M.L. fem. n. *Oligella* referring to small bacterium with limited nutritional properties.

Small rods or coccobacilli, usually not exceeding 1 µm in length and often occurring in pairs; cell width is seldom larger than 0.6 µm. Gram negative, noncapsulated, and nonsporeforming. **Motility, if present, is by means of peritrichous flagella. Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Moderately fastidious chemoorganotrophs growing at 30°C and 37°C. Grow on nutrient agar, but growth is enhanced by the addition of yeast autolysate, serum, or blood. No pigments and no odor are produced. Nonhemolytic. **Biochemically they are rather inert**; only a few organic acids and amino acids are oxidized or utilized as the sole carbon source. **Carbohydrates are neither fermented nor oxidized. Oxidase positive and usually catalase positive.** Indole and H_2S are not formed. Gelatin is not hydrolyzed. The urease test is useful to differentiate the two species. The major cellular fatty acids are *cis*-vaccenic acid ($C_{18:1\ \omega 7c}$) and palmitic acid ($C_{16:0}$); two 3-hydroxylated acids ($C_{14:0\ 3OH}$ and $C_{16:0\ 3OH}$) are also present, whereas branched-chain acids are absent. Mainly isolated from the genitourinary tract of humans. Pathogenicity is unknown but likely low. Belongs to the *Betaproteobacteria*; the genera *Brackiella*, *Pelistega*, and *Taylorella* are phylogenetic neighbors.

The mol% G + C of the DNA is: 46–48.

Type species: **Oligella urethralis** (Lautrop, Bøvre and Frederiksen 1970) Rossau, Kersters, Falsen, Jantzen, Segers, Union, Nehls and De Ley 1987, 206[VP] (*Moraxella urethralis* Lautrop, Bøvre and Frederiksen 1970, 255.)

FURTHER DESCRIPTIVE INFORMATION

Oligella urethralis is nonmotile, whereas most strains of *Oligella ureolytica* are motile by peritrichous flagella. However, motility of *Oligella ureolytica* may be difficult to demonstrate. Colonies on blood agar develop rather slowly and are more overtly white than those of all recognized species of *Moraxella*.

The following descriptive information is mainly based on data from Clark et al. (1984), Schreckenberger and von Graevenitz (1999), Schreckenberger et al., (1999), and, in particular, on the properties of 15 *O. urethralis* strains and 11 *O. ureolytica* strains as reported by Rossau et al. (1987). *Oligella* strains can grow in the presence of up to 3% NaCl. *O. ureolytica* and some strains of *O. urethralis* can also grow in the presence of 4.5% NaCl. Nitrite is reduced. The following physiological features are negative: acid production in oxidative-fermentative (O/F) media containing D-glucose, D-fructose, D-xylose, maltose, and *meso*-ribitol; formation of fluorescent pigment on King B medium; growth on cetrimide; hydrolysis of acetamide, DNA, esculin, gelatin, starch, and Tween 80; lysine and ornithine decarboxylase; and arginine dihydrolase. The following organic compounds are utilized as the sole carbon source (as tested with API auxanographic galleries): acetate, D-alanine, benzoate, fumarate, L-glutamate, glutarate, DL-3-hydroxybutyrate, itaconate, 2-ketoglutarate, DL-lactate, L-malate, pyruvate, succinate, and *n*-valerate. The following compounds are not utilized as carbon source (as tested with API auxanographic galleries): acetamide, *N*-acetylglucosamine, aconitate, adipate, β-

alanine, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, DL-2-aminobutyrate, DL-3-aminobutyrate, DL-4-aminobutyrate, amygdalin, amylamine, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, L-arginine, L-aspartate, azelate, benzylamine, betaine, butylamine, caprate, caprylate, cellobiose, citraconate, L-citrulline, creatine, L-cysteine, diaminobutane, dulcitol, *meso*-erythritol, esculin, ethanolamine, ethylamine, D-fructose, D-fucose, L-fucose, D-galactose, gentiobiose, D-gluconate, D-glucosamine, D-glucose, DL-glycerate, glycerol, glycine, glycogen, glycolate, heptanoate, histamine, L-histidine, *o*-hydroxybenzoate, *meso*-inositol, inulin, 2-ketogluconate, 5-ketogluconate, DL-kynurenine, lactose, levulinate, L-lysine, D-lyxose, maleate, malonate, maltose, D-mandelate, L-mandelate, D-mannitol, D-mannose, melezitose, melibiose, L-methionine, methyl-α-D-glucoside, methyl-α-D-mannoside, methyl-β-D-xyloside, L-ornithine, oxalate, pelargonate, L-phenylalanine, phthalate, *iso*-phthalate, pimelate, raffinose, L-rhamnose, *meso*-ribitol (adonitol), D-ribose, salicin, sarcosine, sebacate, L-serine, sorbitol, L-sorbose, spermine, starch, suberate, sucrose, D-tagatose, D-tartrate, *meso*-tartrate, terephthalate, L-threonine, trehalose, trigonelline, tryptamine, D-tryptophan, L-tryptophan, turanose, L-tyrosine, urea, *meso*-xylitol, D-xylose, and L-xylose. The following substrates are hydrolyzed (as tested with API-ZYM galleries): L-leucyl-2-naphthylamide, 2-naphthyl butyrate, and 2-naphthyl caprylate. The following substrates are not hydrolyzed (using API-ZYM galleries): *N*-benzoyl-DL-arginine-2-naphthylamide, 6-bromo-2-naphthyl-α-D-galactopyranoside, 6-bromo-2-naphthyl-α-D-mannopyranoside, 6-bromo-2-naphthyl-β-D-glucopyranoside, L-cystyl-2-naphthylamide, *N*-glutarylphenylalanine-2-naphthylamide, naphthol-AS-BI-β-D-glucuronate, 1-naphthyl-*N*-acetyl-β-D-glucosaminide, 2-naphthyl-α-L-fucopyranoside, 2-naphthyl-β-D-galactopyranoside, 2-naphthyl-α-D-glucopyranoside, 2-naphthylmyristate, 2-naphthylphosphate (at pH 5.4), and L-valyl-2-naphthylamide.

According to version 3.5 of the Biolog database, the following substrates of the Biolog GN Microplate yield positive results with *Oligella* strains: acetic acid, alaninamide, D-alanine, L-alanine, bromosuccinic acid, formic acid, L-glutamic acid, 2-hydroxybutyric acid, 3-hydroxybutyric acid, itaconic acid, 2-ketobutyric acid, 2-ketoglutaric acid, 2-ketovaleric acid, DL-lactic acid, L-leucine, methylpyruvate, monomethylsuccinate, L-proline, propionic acid, succinamic acid, and succinic acid.

The major cellular fatty acids of *Oligella* are *cis*-vaccenic acid ($C_{18:1\ \omega7c}$) and palmitic acid ($C_{16:0}$) (comprising respectively approximately 35–45% and 20–25% of the total fatty acid content). Two 3-hydroxylated acids $C_{14:0\ 3OH}$ and $C_{16:0\ 3OH}$ are present (3–5%), whereas branched chain acids are reported to be absent (Rossau et al., 1987; Moss et al., 1988; Schreckenberger and von Graevenitz, 1999). Only *O. ureolytica* strains characteristically possess in addition lactobacillic acid ($C_{19:0\ cyclo\ \omega8c}$) (Rossau et al., 1987). However, when cultivated on tryptic soy medium, no $C_{19:0\ cyclo\ \omega8c}$ was detected for the type strain of *O. ureolytica* (M. Vancanneyt, unpublished data).

O. urethralis contains ubiquinone with eight isoprene units as the major isoprenolog (Moss et al., 1988).

O. urethralis produces 2-hydroxyputrescine and putrescine as predominant polyamine compounds (respectively 22 and 10 μmol/dry weight), and smaller amounts of spermidine (4 μmol/g dry weight) (H.-J. Busse, personal communication).

Oligella urethralis strains are generally susceptible to most antibiotics, including penicillin, whereas *O. ureolytica* is susceptible to a limited number of antibiotics (Welch et al., 1983). Quinolone resistance has been reported in *O. urethralis* (Riley et al., 1996).

The size of *Oligella* genomes is 1.2–1.4 $\times$ 10^9 Da (Rossau et al., 1987).

Oligella strains have been mainly isolated from the human genitourinary tract, and both species have been reported to cause urosepsis (Rockhill and Lutwick, 1978; Pugliese et al., 1993). Some strains of *O. urethralis* were also isolated from the ear, blood, and a foot wound. Cases of septic arthritis and chronic ambulatory peritoneal dialysis peritonitis caused by *O. urethralis* have been reported (Mesnard et al., 1992; Riley et al., 1996). *O. urethralis* has also been isolated from the conjunctiva of rabbits (Marini et al., 1996).

ENRICHMENT AND ISOLATION PROCEDURES

The nutritional requirements of *Oligella* have not been studied in detail. Strains grow on ordinary blood media, heart infusion agar, tryptic soy agar, and Drigalski agar. Incubation in the presence of 5% CO_2 will usually enhance growth. Growth on MacConkey agar is variable. The majority of the *O. urethralis* strains will also grow on nutrient agar. No procedures for the selective isolation of *Oligella* have been worked out.

MAINTENANCE PROCEDURES

Oligella strains can be maintained by classical preservation techniques such as lyophilization, storage in liquid nitrogen, and storage in a freezer at $-80°C$.

DIFFERENTIATION OF THE GENUS *OLIGELLA* FROM OTHER GENERA

Table BXII.β.35 summarizes the differential characteristics between *Oligella* and the phylogenetically closely related genera *Pelistega* and *Taylorella*, as well as the phenotypically similar species *Bordetella bronchiseptica*, *Moraxella osloensis*, and *Ralstonia paucula*, a new species encompassing bacteria of the CDC group IVc-2 (Vandamme et al., 1999).

Rossau et al. (1987), Holt et al. (1994), Weyant et al. (1996), Schreckenberger et al. (1999), and Schreckenberger and von Graevenitz (1999) have compiled other useful tables for differentiating *Oligella* from morphologically or biochemically similar bacterial taxa such as *Moraxella*. Gas chromatographic analysis of the cellular fatty acids yields usually a good discrimination between these taxa; e.g., *Oligella* cells possess $C_{18:1\ \omega7c}$ as the major acid, whereas authentic *Moraxella* species lack this compound, and typically contain oleic acid ($C_{18:1\ \omega9c}$) (Moss et al., 1988) (Table BXII.β.35). The biochemical inertness of the *Oligella* strains renders the identification of *Oligella* and phenotypically similar species difficult. Due to its fast and strong urease reaction, *O. ureolytica* can be confused with *B. bronchiseptica* or *Ralstonia paucula* (CDC group IVc-2) (Table BXII.β.35).

TAXONOMIC COMMENTS

The genus *Oligella* currently contains two species: *Oligella urethralis*, which was previously classified in the genus *Moraxella*, and *Oligella ureolytica*, which was formerly referred to as CDC group IVe bacteria. Based on comparative 16S rDNA-analysis *Oligella* belongs to the *Betaproteobacteria*, its closest phylogenetic neighbors are the genera *Brackiella*, *Pelistega*, and *Taylorella* (see Fig. BXII.β.25 in this chapter and Fig. BXII.β.21 in the chapter on the family *Alcaligenaceae*).

Oligella urethralis was first described as *Moraxella urethralis* by Lautrop et al. (1970), who stated that the allocation of these bacteria in the genus *Moraxella* was only a matter of convenience. Hence in the previous edition of *Bergey's Manual*, *M. urethralis* was considered as a *species incertae sedis* of the genus *Moraxella*

TABLE BXII.β.35. Differential characteristics of the genus *Oligella* and phylogenetically related or phenotypically similar bacteria[a,b]

Feature	*Oligella*[c]	*Pelistega europaea*[d]	*Taylorella equigenitalis*[e]	*Ralstonia paucula* (CDC group IVc-2)[f]	*Bordetella bronchiseptica*[g]	*Moraxella osloensis*[h]
Cell diameter, μm	0.6	0.2–0.4	0.7	0.8	0.2–0.5	1.0–1.5
Cell length, μm	<1.0	1.0–2.0	0.7–1.8	1.2–2.0	0.5–2.0	1.5–2.5
Motility (peritrichous flagella)	D	−	−	+	+	−
Growth at 42°C	D	+	+	+	+	−
Growth on MacConkey agar	d	−	−	+		d
Growth in presence of 3% NaCl	+			−		−
Urease	D	d	−	+	+	−
Nitrate reduction	D	−	−	−	+	d
Nitrite reduction	+	−	−	−	−	−
Denitrification	d	−	−	−	−	−
Carbon sources for growth:						
Adipate	−	−		+	+	−
D-Gluconate	−	−		+	−	−
Benzoate	+			d	−	−
p-Hydroxybenzoate	D			−	−	−
L-Malate	+	+		+	+	−
Characteristic cellular fatty acids[i]:						
C$_{10:0}$						+
C$_{12:0}$		+				
C$_{14:0}$	+	+		+	+	
C$_{16:0}$	+	+	+	+	+	+
C$_{16:1}$	+		+	+		
C$_{18:0}$			+		+	+
C$_{16:1\ \omega7c}$		+			+	+
C$_{17:0\ cyclo}$				+	+	
C$_{18:1\ \omega7c}$	+	+	+	+	+	
C$_{18:1\ \omega9c}$						+
C$_{12:0\ 2OH}$					+	
C$_{12:0\ 3OH}$						+
C$_{14:0\ 3OH}$	+		+			
C$_{16:0\ 3OH}$	+	+	+	+		
C$_{16:1\ 2OH}$				+		
C$_{18:1\ 2OH}$				+		
SF 3[j]		+		+	+	
Mol% G + C of DNA	46–48	42–43	36–38	65–67	68–69.5	43–46

[a]Symbols: see standard definitions.

[b]Data from Clark et al. (1984), Rossau et al. (1987), Moss et al. (1988), Vandamme et al. (1998, 1999), and Schreckenberger and von Graevenitz (1999).

[c]Isolated from human genitourinary tract.

[d]Isolated from respiratory tracts of pigeons.

[e]Isolated from genital tract of mares; caused endometritis and cervicitis.

[f]Isolated from various human clinical and environmental sources.

[g]Isolated from respiratory tract of animals and humans.

[h]Isolated from clinical sources.

[i]The references for the fatty acid data are as follows: *Oligella* (Rossau et al., 1987), *Pelistega* (Vandamme et al., 1998), *Taylorella* (Rossau et al., 1987), *Ralstonia paucula* (P. Vandamme, personal communication), *Bordetella bronchiseptica* (Vancanneyt et al., 1995), and *Moraxella osloensis* (Moss et al., 1988; Vandamme et al., 1993b).

[j]SF 3 (summed feature 3) comprises C$_{14:0\ 3OH}$ or C$_{16:1\ iso\ I}$, or any combination of these fatty acids.

(Bøvre, 1984b), which is phylogenetically quite distinct from *Oligella* (Fig. BXII.β.25); the authentic *Moraxella* species belong to the *Gammaproteobacteria* (Rossau et al., 1991; Pettersson et al., 1998b). Although *O. urethralis* displays several phenotypic features in common with *Moraxella* species (particularly *M. osloensis*), genetic transformation studies with streptomycin resistance as a marker indicated that the species *Oligella urethralis* (previously named *Moraxella urethralis*) displayed no distinct genetic affinities to the authentic *Moraxella* species (Bøvre, 1980). Consequently, Bøvre (1984b) suggested that the species might belong to a separate genus. Moreover, *Oligella* cells are also smaller and lack the typical plumpness of moraxellae, and their cellular fatty acid composition differs significantly (Moss et al., 1988; Table BXII.β.35). *O. urethralis* was formerly also referred to as "CDC group M-4", and includes some of the strains previously called "*Mima polymorpha* biovar oxidans".

O. ureolytica was previously known as CDC group IVe, displaying some phenotypic similarities (e.g., rapid and strong urease test) with *Bordetella bronchiseptica* and *Ralstonia paucula* (formerly CDC group IVc-2) (Clark et al., 1984; Vandamme et al., 1999).

The grouping of *Oligella* in the *Betaproteobacteria* was initially established by Rossau et al. (1987) based on rRNA–DNA hybridization studies. Recently the knowledge of the phylogenetic relationships of the genus has been refined by an analysis of the nearly complete 16S rDNA sequences of the type strains of the species *O. urethralis* and *O. ureolytica* (M. Vancanneyt and B. Hoste, unpublished data) (Fig. BXII.β.25). The type strains of the two *Oligella* species display a significant 16S rDNA similarity (97%). The nearest phylogenetic neighbors of *Oligella* are *Pelistega europaea* (92.1%) and *Taylorella equigenitalis* (91.8%) (Fig. BXII.β.25). At present *Pelistega* encompasses one species, *P. europaea* (Vandamme et al., 1998), which is involved in the path-
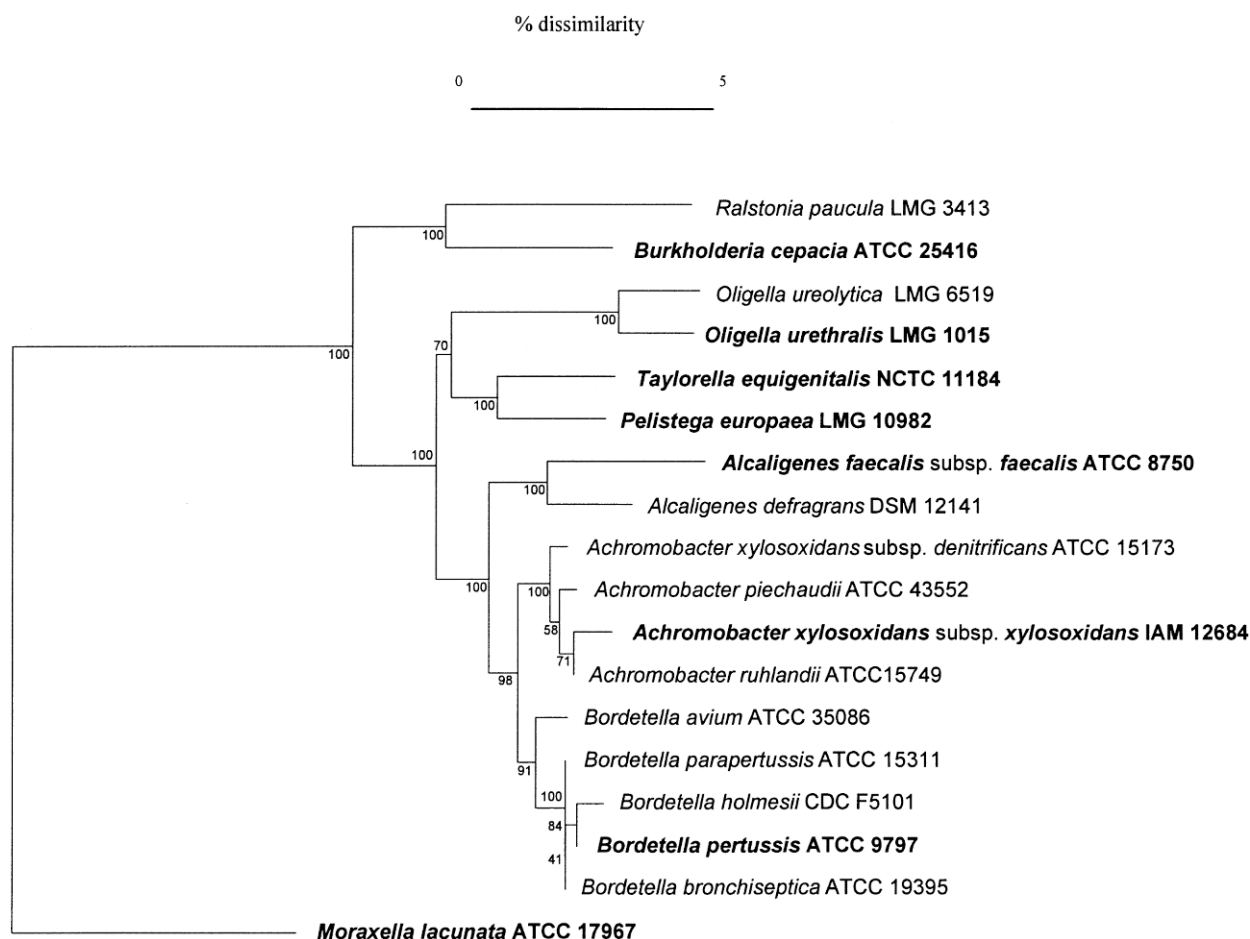
% dissimilarity

0   5

FIGURE BXII.β.25. Phylogenetic tree of members of the genus *Oligella* and related bacteria. The analysis was performed using the software package GeneCompar (Applied Maths, Kortrijk, Belgium) after including the consensus sequence in a multiple alignment of small ribosomal subunit sequences collected from the international nucleotide sequence library EMBL. A resulting tree was constructed using the neighbor-joining method. Unknown bases and gaps were not considered in the numerical analysis. Bootstrap probability values are indicated at the branch-points (100 trees resampled). All strains are type strains and the type species are indicated in bold.

ogenesis of respiratory diseases in pigeons. *Taylorella equigenitalis*, the sole species of the genus *Taylorella* (Sugimoto et al., 1983) is the causative agent of contagious equine metritis, a sexually transmitted bacterial disease of horses. The phylogenetic grouping of *T. equigenitalis* within the *Betaproteobacteria* was reported by Bleumink-Pluym et al. (1993) and Rossau et al. (1987). The 16S rDNA similarity values of *Oligella* versus the investigated type strains of the various species of the family *Alcaligenaceae* vary between 91.3% and 92.6%, whereas similarity values with the type species of the genus *Moraxella* are only 82% (Fig. BXII.β.25). It is surprising that the phylogenetically related taxa *Achromobacter*, *Alcaligenes*, *Bordetella*, *Oligella*, *Pelistega*, and *Taylorella* (Fig. BXII.β.25) cover in their genomic DNA a very broad mol% G + C range (36–70) (Table BXII.β.35).

The majority of the above-mentioned taxa, including *Oligella* but excluding some of the *Bordetella* species, can be circumscribed as oxidase positive, indole-negative, asaccharolytic nonferment-ing Gram-negative bacteria. Strains of the genera *Alcaligenes*, *Achromobacter* (Yabuuchi et al., 1998a), and *Bordetella* can be implicated in human and animal diseases.

FURTHER READING

Rossau, R., K. Kersters, E. Falsen, E. Jantzen, P. Segers, A. Union, L. Nehls and J. De Ley. 1987. *Oligella*, a new genus including *Oligella urethralis* comb. nov. (formerly *Moraxella urethralis*) and *Oligella ureolytica* sp. nov. (formerly CDC group IVe): relationship to *Taylorella equigenitalis* and related taxa. Int. J. Syst. Bacteriol. *37*: 198–210.

Schreckenberger, P.C. and A. von Graevenitz. 1999. *Acinetobacter, Achromobacter, Alcaligenes, Moraxella, Methylobacterium*, and other nonfermentative Gram-negative rods. *In* Murray, Baron, Pfaller, Tenover and Yolken (Editors), Manual of Clinical Microbiology, 7th Ed., ASM Press, Washington D.C. pp. 539–560.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *OLIGELLA*

Characteristics useful in differentiating the species of *Oligella* are given in Table BXII.β.36.

List of species of the genus Oligella

1. **Oligella urethralis** (Lautrop, Bøvre and Frederiksen 1970) Rossau, Kersters, Falsen, Jantzen, Segers, Union, Nehls and De Ley 1987, 206[VP] (*Moraxella urethralis* Lautrop, Bøvre and Frederiksen 1970, 255.)

   u.re.thra' lis. Gr. *ourethra* urethra; M.L. gen. n. *urethralis* of the urethra.

   The description is as given for the genus and as listed in Tables BXII.β.35 and BXII.β.36. Cells are nonmotile. Inclusions of intracellular poly-β-hydroxybutyrate have been reported. Grows at 42°C. Urease negative (Christensen's).

TABLE BXII.β.36. Differential features of the species of the genus *Oligella*[a,b]

Characteristic	*O. urethralis*	*O. ureolytica*
Motility	−	d
Growth at 42°C	+	−
Growth in the presence of 4.5% NaCl	d	+
Urease	−	+[c]
Nitrate reduction	−	d
Denitrification	+[d]	d[d]
Growth on:		
p-Hydroxybenzoate	−	+
DL-5-Aminovalerate	+	−
Presence of $C_{19:0 \text{ cyclo}}$ fatty acid (>1%)	−	+

[a]Symbols: see standard definitions.

[b]Data from Clark et al. (1984), Rossau et al. (1987), and Schreckenberger and von Graevenitz (1999).

[c]The urease reaction is strongly positive.

[d]Demonstration of gas may be difficult.

No growth on p-hydroxybenzoate. Nitrate is not reduced, but denitrification is usually positive. Usually highly sensitive to penicillin. Lactobacillic acid ($C_{19:0 \text{ cyclo } \omega 8c}$) is not detectable among the cellular fatty acids.

Has been isolated from human urine, the human genitourinary tract, and the ear.

The mol% G + C of the DNA is: 46–47.5 (T_m).

Type strain: ATCC 17960, CCUG 13463, CDC 7603, DSM 7531, LMG 5303.

GenBank accession number (16S rRNA): AF133538, AF227163.

2. **Oligella ureolytica** Rossau, Kersters, Falsen, Jantzen, Segers, Union, Nehls and De Ley 1987, 209[VP]

   ur' e.o.ly.ti.ca. M.L. n. *urea* urea; Gr. adj. *lyticus* dissolving; M.L. adj. *ureolytica* dissolving (hydrolyzing) urea.

   The description is as given for the genus and as listed in Tables BXII.β.35 and BXII.β.36. The species accommodates the CDC group IVe strains.

   Some strains are motile by means of long peritrichous flagella. No growth at 42°C. Grows on p-hydroxybenzoate as carbon source. The urease test (Christensen's) is usually strongly positive after 4 h. Most strains reduce nitrate and are resistant to penicillin. Among the cellular fatty acids lactobacillic acid ($C_{19:0 \text{ cyclo } \omega 8c}$) is present in moderate amounts.

   Isolated from human urine, particularly from males.

   The mol% G + C of the DNA is: 46–47 (T_m).

   Type strain: ATCC 43534, CCUG 1465, CDC C379, LMG 6519.

   GenBank accession number (16S rRNA): AJ251912.

Genus VI. **Pelistega** *Vandamme, Segers, Ryll, Hommez, Vancanneyt, Coopman, De Baere, Van De Peer, Kersters, De Wachter and Hinz 1998, 437[VP]*

PETER VANDAMME AND KARL-HEINZ HINZ

Pe.li' ste.ga. Gr. n. *peleia* pigeon; Gr. fem. n. *stege* house, stay, residence; N.L. fem. n. *Pelistega* refers to the bacteria living in pigeons.

Cells in 16–24-h-old cultures on blood agar are 0.2–0.4 × 1–2 μm, with **variable morphological forms**. Gram negative. **Nonsporeforming.** Capsulated. **Nonmotile. Optimal growth under microaerobic conditions.** Growth is weak under aerobic conditions and absent under anaerobic conditions. Growth at 37°C and 42°C, not at 24°C. Does not require growth factors on conventional media. No growth on MacConkey agar. **Glucose is oxidized with the production of alkali**, no fermentation of, or acid production from, glucose. **Catalase and oxidase activity is present.** No reduction of nitrate, no denitrification, no production of acetylmethylcarbinol, indole, or methyl red, no esculin hydrolysis, β-galactosidase, DNase, chondroitin sulfatase, hyaluronidase, lysine decarboxylase, ornithine decarboxylase, lecithinase, or phenylalanine deaminase activity. **Alkaline and acid phosphatase activity is present.** Thus far only isolated from pigeon samples.

The mol% G + C of the DNA is: 42–43.

Type species: **Pelistega europaea** Vandamme, Segers, Ryll, Hommez, Vancanneyt, Coopman, De Baere, Van De Peer, Kersters, De Wachter and Hinz 1998, 437.

FURTHER DESCRIPTIVE INFORMATION

The genus *Pelistega* belongs to the *Betaproteobacteria* with the genus *Taylorella* as its closest relative (Fig. BXII.β.26). Other genera belonging to the same phylogenetic neighborhood are *Bordetella*, *Alcaligenes*, and *Burkholderia*.

Strains of the genus *Pelistega* have been isolated from samples of lungs, air sac exudate, and trachea mucosa of pigeons; less frequently from other organs such as the liver and spleen. The pathogenicity is unknown.

ENRICHMENT AND ISOLATION PROCEDURES

At present, blood agar is the only medium by which *Pelistega* strains have been successful isolated. MacConkey agar and litmus
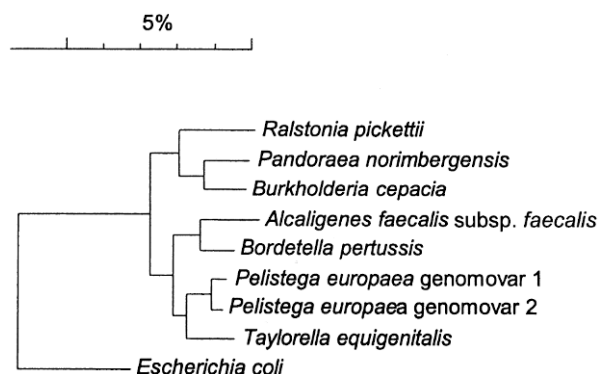
FIGURE BXII.β.26. Phylogeny of *Pelistega europaea*. Bar = 5% difference in 16S rRNA nucleotide sequences. The neighbor-joining method was used for tree construction. Accession numbers for the sequences used are: *Alcaligenes faecalis* subsp. *faecalis* LMG 1229^T, D88008; *Bordetella pertussis* LMG 14455^T, U04950; *Burkholderia cepacia* LMG 1222^T, M22518; *Pandoraea norimbergensis* LMG 18379^T, Y09879; *Pelistega europaea* genomovar 1 LMG 10982^T, Y11890 and *Pelistega europaea* genomovar 2 LMG 15725, AF190911; *Ralstonia pickettii* LMG 5942^T, X67042; *Taylorella equigenitalis* LMG 6222^T, X68645; *Escherichia coli* (J01695) was chosen as an outgroup.

lactose agar are not suitable for primary isolation. Selective procedures have not been developed. Clinical specimens are plated on blood agar plates and incubated at 37°C for 24–48 h in a microaerobic atmosphere with a high humidity (e.g., candle jar conditions). *Pelistega* strains can be identified especially by the characters given in Table BXII.β.37. The numerical analysis of the whole-cell fatty acid patterns by using the MIDI system also allows a rapid and reliable identification.

MAINTENANCE PROCEDURES

Cultures may be stored for many years by lyophilization, freezing at −80°C, or in liquid nitrogen. Cryoprotective agents such as 10% glycerol or DMSO should be added to cultures before freezing. Strains also remain viable for over 10 years when stored as dense suspensions in skimmed milk in a frozen state at −70°C. Twenty-four-hour blood agar cultures will survive refrigeration (4°C) in ambient air for at least 1 week.

DIFFERENTIATION OF THE GENUS *PELISTEGA* FROM OTHER GENERA

Comparative 16S rDNA sequence analysis revealed that *Pelistega europaea* belongs to the *Betaproteobacteria* with *Taylorella equigenitalis* as its nearest neighbor (Fig. BXII.β.26). Like *Pelistega europaea*, *Taylorella equigenitalis* (the type species of the genus *Taylorella*) is a microaerobic, Gram-negative, nonmotile, rod-shaped organism that does not acidify carbohydrates and exhibits catalase, oxidase, and acid and alkaline phosphatase activity. *Taylorella equigenitalis* differs from *Pelistega europaea* by the absence of urease and arginine dihydrolase activity, and by alkali production from glucose. *Taylorella equigenitalis* strains can also be differentiated from *Pelistega europaea* by means of whole-cell protein and fatty acid analysis and by its mol% G + C content (about 36–37% for the former and 42–43% for the latter). The most salient differences between *Pelistega europaea* and *Taylorella equigenitalis*, however, are the fastidious growth requirements of the latter, while *Pelistega europaea* grows abundantly when incubated under microaerobic conditions. Vandamme et al. (1998) reported a remarkable stim-

ulation of growth of *Taylorella equigenitalis* strains by the addition of growth factors, which was not recorded for *Pelistega europaea* strains. Earlier studies by Sugimoto et al. (1983), however, did not reveal this growth stimulation.

Table BXII.β.37 lists differential diagnostic characteristics that can be used to distinguish *Pelistega europaea* from other species that may be encountered in specimens from pigeons: *Riemerella anatipestifer*, *Ornithobacterium rhinotracheale*, *Chryseobacterium meningosepticum*, and *Pasteurella* species.

TAXONOMIC COMMENTS

Vandamme et al. (1998) described a polyphasic taxonomic study of a collection of 24 pigeon isolates that remained unidentified after preliminary examination by conventional biochemical tests. Additional extensive biochemical characterization by using a considerable number of conventional tests and several API microtest systems further substantiated the high phenotypic similarity among all of the strains examined. Unexpectedly, whole-cell fatty acid analysis demonstrated a marked subdivision of the pigeon isolates into two main clusters. Strains of both clusters had the same major fatty acid components but there were marked quantitative differences. However, the same strains were again further subdivided into four subgroups and several strains with unique patterns by means of one-dimensional whole-organism protein electrophoresis. The DNA base ratio of representative strains of these subgroups was determined, and all values were between 42–43 mol% G + C. Results obtained by whole-cell protein electrophoresis were a good indicator of the level of DNA–DNA hybridization, as only within the protein electrophoretic subgroups high binding levels were detected (>82%); all other values were low or insignificant.

In taxonomic practice, the DNA–DNA hybridization level within a bacterial species is mostly above 50–70%. This level is primarily determined by the phenotypic consistency of the taxon studied, as species should be identifiable by phenotypic characteristics (Wayne et al., 1987; Ursing et al., 1995). The term "genomovar" refers to closely related genomic species that cannot be distinguished by phenotypic characteristics (Ursing et al., 1995). The group of pigeon isolates obviously comprised several subgroups that were genotypically distinct enough to warrant their classification as different species. However, in the absence of differential phenotypic characteristics between these genomovars, all isolates were included in a single nomenspecies, *Pelistega europaea*.

Strain LMG 10982 (representing genomovar 1) was chosen as the type strain for the nomenspecies. Strains LMG 15725, LMG 11609, and LMG 12985 were selected as reference strains for genomovars 2, 3, and 4, respectively. In addition, several strains with unique whole-organism protein patterns were not formally classified into different genomovars until the DNA–DNA hybridization levels between these strains and the four designated genomovars were fully determined.

It therefore should be clear that *Pelistega europaea* presently encompasses multiple genomic species. It is not unlikely that this name may be restricted in the future to only one or a few of these genomovars, upon discovery of differential diagnostic features.

TABLE BXII.β.37. Differential characteristics between *Pelistega europaea* and other bacteria from pigeons[a]

Characteristic	*Pelistega europaea*	*Chryseobacterium meningosepticum*	*Ornithobacterium rhinotracheale*	*Pasteurella* species	*Riemerella columbina*
Pigmentation	−	V[b]	−	−	−
Growth on MacConkey agar	−	+	−	−	−
Aerobic growth on blood agar at 37°C	+ or W	+	W	+	+
Catalase activity	+	+	−	+	+
Urease activity	V	−	+	−	V
Nitrate reduction	−	−	−	+	−
Indole production	−	V[b]	−	V	−
Gelatinase activity	−	+	−	−	+
Chondroitin sulfatase activity	−	ND	+	V	−
Hyaluronidase activity	−	ND	+	V	−
Esculin hydrolysis[c]	−	+	−	−	+
β-Galactosidase activity	−	+	+	V[b]	−
Acid production from glucose	−	V[b]	+	+	+
Utilization of carbon sources for growth[c]	+	+	−	−	−
DNA base ratio	42–43	36–37	37–39	40–45	36–37
Host spectrum	Pigeons	Humans, birds	Turkeys, chickens, wild birds	Mammals, birds	Pigeons

[a]Symbols: +, characteristic present; −, characteristic absent; V, strain-dependent reaction; W, weak reaction; ND; not determined.

[b]Over 80% of the strains contains this feature.

[c]As determined using the API 20 NE microtest system.

FURTHER READING

Sugimoto, C., Y. Isayama, R. Sakazaki and S. Kuramochi. 1983. Transfer of *Haemophilus equigenitalis* Taylor et al., 1978 to the genus *Taylorella*, gen. nov. as *Taylorella equigenitalis*, comb. nov. Curr. Microbiol. *9*: 155–162.

Vandamme, P., P. Segers, M. Ryll, J. Hommez, M. Vancanneyt, R. Coopman, R. de Baere, Y. van de Peer, K. Kersters, R. De Wachter and K.H. Hinz. 1998. *Pelistega europaea* gen. nov., sp. nov., a bacterium associated with respiratory disease in pigeons: taxonomic structure and phylogenetic allocation. Int. J. Syst. Bacteriol. *48*: 431–440.

List of species of the genus Pelistega

1. **Pelistega europaea** Vandamme, Segers, Ryll, Hommez, Vancanneyt, Coopman, De Baere, Van De Peer, Kersters, De Wachter and Hinz 1998, 437[VP]

eu.ro.pae' a. L. adj. *europaea* of Europe, because the first collection of strains was isolated in different European countries.

Cells in 16–24-h-old cultures on blood agar are 0.2–0.4 × 1–2 μm, with variable morphological forms. Gram negative. Nonsporeforming. Capsulated. Nonmotile.

Strains produce convex, circular, and grayish-white to yellowish colonies with entire edge and smooth surface on blood agar.

Optimal growth under microaerobic conditions. Growth is weak under aerobic conditions, and absent under anaerobic conditions. Growth at 37°C and 42°C, not at 24°C. Does not require growth factors on conventional media. In standard II nutrient broth, growth appears as moderate turbidity and pellicle near the surface of the broth after 24–48 h of incubation. In most cases, a small, button-like sediment could be seen. No growth on litmus lactose agar at the primary isolation from the infected tissues. However, after some passages on artificial media some strains showed a poor growth. No growth on MacConkey agar.

All strains produce catalase and oxidase activity; urease and arginine dihydrolase activity is strain dependent. No reduction of nitrate, no denitrification, no production of acetylmethylcarbinol, indole, or methyl red, no esculin hydrolysis, gelatinase (except strain LMG 11609), β-galactosidase, DNase, chondroitin sulfatase, hyaluronidase, lysine decarboxylase, ornithine decarboxylase, lecithinase, or phenylalanine deaminase activity. Glucose is oxidized with the production of alkali, no fermentation of, or acid production from, glucose. Malonate is not used as a carbon source. Strain-dependent utilization of citrate on Simmons' citrate agar.

No acid production from D-glucose, D-fructose, sucrose, lactose, maltose, D-galactose, galacturonate, D-mannose, rhamnose, cellobiose, palatinose, dextrin, *N*-acetyl-D-glucosamine, lactulose, L-sorbose, adonitol, D-mannitol, L-arabinose, D- and L-arabitol, salicin, D-sorbitol, trehalose, D-xylose, dulcitol, inositol, or *myo*-inositol.

There is no assimilation of D-glucose, maltose, D-mannose, *N*-acetyl-D-glucosamine, D-mannitol, L-arabinose, D-gluconate, caprate, adipate, or phenyl acetate; most strains (18 out of 19 examined) assimilate L-malate.

Alkaline and acid phosphatase, esterase C_4, ester lipase C_8, and leucine, arginine, alanine, glycine, and L-aspartic acid arylamidase activity is present. Activity of naphthol-AS-Bl-phosphohydrolase and lipase (as present in the API ID 32E system) is strain dependent. Lipase C_{14}, valine, leucyl glycine, phenylalanine, histidine, glutamyl glutamic acid, serine, and cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, α-maltosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activity has not been detected. Proline, pyroglutamic acid, and tyrosine arylamidase activity is strain dependent.

Two major clusters of strains are delineated by means of cellular fatty acid analysis. The major fatty acid components of all strains examined were $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1 \, \omega 7c}$, $C_{16:1 \, \omega 5c}$, $C_{16:0 \, 3OH}$, and summed features 3 and 7. However, the percentages of these fatty acids vary strongly among the different genomovars (Table BXII.β.38).

Strains have been isolated on common nonselective blood agar media under microaerobic (candle jar) conditions mainly from samples of lungs, air sac exudate, and

TABLE BXII.β.38. Fatty acid composition of *Pelistega europaea* strains[a]

Fatty acid	Genomovars 1, 2, and 3	Genomovar 4
$C_{12:0}$	4.4 ± 0.7	4.4 ± 1.0
$C_{14:0}$	9.7 ± 1.5	10.8 ± 2.5
$C_{16:0}$	15.7 ± 2.9	25.2 ± 2.8
$C_{18:0}$	Tr	Tr
$C_{16:1\ \omega7c}$	21.7 ± 3.5	41.4 ± 2.7
$C_{16:1\ \omega5c}$	5.2 ± 2.6	Tr
$C_{16:0\ 3OH}$	1.2 ± 0.3	1.2 ± 0.2
$C_{19:0\ 10CH3}$	Tr	nd
Summed feature 1[b]	Tr	2.7 ± 1.6
Summed feature 3[c]	12.7 ± 2.1	12.4 ± 1.1
Summed feature 7[d]	27.8 ± 9.4	1.5 ± 0.9

[a]Those fatty acids for which the average amount for all taxa was less than 1% are not given. Therefore, the percentages for each group do not total 100%. Mean percentages and standard deviations are given; Tr, trace amount (less than 1%); nd, not detected.

[b]Summed feature 1 comprises $C_{14:1\ \omega5c}$, $C_{14:1\ \omega5t}$, or both.

[c]Summed feature 3 comprises $C_{14:0\ 3OH}$, $C_{16:1\ iso\ I}$, an unidentified fatty acid with equivalent chain length value of 10.928, or 12:0 ALDE, or any combination of these fatty acids.

[d]Summed feature 7 comprises $C_{18:1\ \omega7c}$, $C_{18:1\ \omega9t}$, or $C_{18:1\ \omega12t}$, or any combination of these fatty acids.

trachea mucosa, and less frequently from other organs such as the liver and spleen. They also can be isolated from swabs taken from the palatine cleft or trachea of living acutely diseased pigeons. At present the knowledge with regard to pathogenicity is incomplete. The present clinical observations suggest that they are pathogenic and involved especially in pathogenesis of respiratory diseases in pigeons. However, the role of cofactors and the interaction with other agents are uncertain. Bacteria associated with similar clinical signs in pigeons were described by Andreasen and Sandhu (1993). All morphological and biochemical characteristics reported by the latter authors corresponded with our findings.

The type strain was isolated from a pigeon in Belgium.

The mol% G + C of the DNA is: 42–43 (43 for the type strain) (T_m).

Type strain: Strain Hommez N57, LMG 10982.

GenBank accession number (16S rRNA): Yl1890.

Additional Remarks: GenBank accession number (16S rRNA) for LMG 15725 (reference strain for genomovar 2) is AF190911.

Genus VII. **Pigmentiphaga** Blümel, Mark, Busse, Kämpfer and Stolz 2001b, 1870[VP]

Hans-Jürgen Busse

Pig.men.ti.pha.ga. L. n. *pigmentum* dye; Gr. n. *phagos* eater; M.L. fem. adj. *Pigmentiphaga* eating dyes.

Cells are Gram-negative, motile, rod-shaped, nonsporeforming bacteria 1.3–4 × 0.7–1.2 μm. The colonies are opaque, circular, convex with an entire margin. Growth occurs at 30°, 37°, and 42°C, no growth is found at 4°C.

Oxidase and catalase positive. L-Alanine-*p*-nitroanilide is hydrolyzed. The following compounds are not hydrolyzed: *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-glucuronide, *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-β-D-xylopyranoside, bis-*p*-nitrophenyl-phosphate, bis-*p*-nitrophenyl-phenyl-phosphonate, bis-*p*-nitrophenyl-phosphoryl-choline, L-aniline-*p*-nitroanilide, γ-L-glutamate-*p*-nitroanilide, and L-proline-*p*-nitroanilide. The following compounds are assimilated: acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, mesaconate, 2-oxoglutarate, pyruvate, suberate, L-aspartate, 3-hydroxybenzoate, and 4-hydroxybenzoate. The following compounds are not assimilated: *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-maltose, D-mannose, α-D-melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, D-trehalose, D-xylose, adonitol, *i*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, 4-aminobutyrate, L-alanine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan, and phenylacetate. No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol, and D-mannose.

The **major fatty acids** are $C_{16:0}$, **summed feature 3** ($C_{16:1\ iso\ I}$ and $C_{14:0\ 3OH}$), **summed feature 7** ($C_{18:1\ \omega7c}$, $C_{18:1\ \omega9t}$, and/or $C_{18:1\ \omega12t}$), **and $C_{10:0\ 3OH}$, $C_{14:0\ 2OH}$, $C_{16:0\ 2OH}$, and $C_{17:0\ cyclo}$ and $C_{19:0\ cyclo\ \omega8c}$**; the major isoprenoid quinone is **ubiquinone Q-8**. The **polyamine pattern** contains two major compounds: **putrescine** and the unusual **2-hydroxyputrescine**. **Phosphatidylethanolamine** is the major **polar lipid** and **phosphatidylglycerol** and **diphosphatidylglycerol** are minor components.

The mol% G + C of the DNA is: 68.5 ± 0.3.

Type species: **Pigmentiphaga kullae** Blümel, Mark, Busse, Kämpfer and Stolz 2001b, 1870.

List of species of the genus Pigmentiphaga

1. **Pigmentiphaga kullae** Blümel, Mark, Busse, Kämpfer and Stolz 2001b, 1870[VP]

   kul.lae. in honor of Hans G. Kulla, who initiated the work about the aerobic degradation of azo dyes by bacteria, which resulted in the isolation of strain K24.

   The characteristics of the species is identical to that of the genus. Strain K24[T] has been isolated from soil after continuous enrichment with 1-(4′-carboxyphenylazo)-4-naphthol.

   The mol% G + C of the DNA is: 68.5 ± 0.3 (HPLC).

   Type strain: K24, DSM 13608, NCIMB 13708.

   GenBank accession number (16S rRNA): AF282916.

Genus VIII. **Sutterella** Wexler, Reeves, Summanen, Molitoris, McTeague, Duncan, Wilson and Finegold 1996a, 257^VP

HANNAH M. WEXLER

Sut.ter.el' la. M.L. dim. fem. n. *Sutterella* named in memory of Vera Sutter, respected colleague and director of the Wadsworth Anaerobe Laboratory for twenty years.

Straight rod, 0.5–1 × 1–3 μm. Gram negative. Grows in a microaerophilic atmosphere (2% and 6% oxygen) or under anaerobic conditions. Isolated mainly from the intestinal tract and from infections of gastrointestinal origin. Urease negative. Oxidase negative. Indoxyl acetate negative. Reduces nitrate. **Resistant to 20% bile disks.** Asaccharolytic. Cannot reduce tetrazolium tetrachloride under aerobic conditions if formate and fumarate are added to the medium. **May be resistant to metronidazole.**

Type species: **Sutterella wadsworthensis** Wexler, Reeves, Summanen, Molitoris, McTeague, Duncan, Wilson and Finegold 1996a, 257.

FURTHER DESCRIPTIVE INFORMATION

Biochemical characteristics and oxygen tolerance Like *B. ureolyticus, C. gracilis,* and other *Campylobacter* species, members of *Sutterella* are asaccharolytic and reduce nitrate to nitrite. Both *Campylobacter* and *Sutterella* were able to grow on solid media at 2% and 6% oxygen. *C. gracilis* grew only in 6% oxygen on plates containing *Brucella* agar with formate/fumarate but no blood added; if blood was added, *C. gracilis* did not grow. *Sutterella* is resistant to both human bile and oxgall (Table BXII.β.39); this may be important in their ability to survive in the biliary tract and bowel.

Dehydrogenase profiles Three general patterns of dehydrogenase activity were seen among the typical *C. gracilis, Sutterella wadsworthensis,* and *Campylobacter* strains studied. The group including the type strain of *C. gracilis* (as well as *C. gracilis* type 2 WAL 10733/FDC 20A1) showed no bands or very faintly reactive malate-specific bands (which appeared only when the gels were very heavily loaded), whereas strains of *Sutterella* had strongly reactive single or multiple bands of a nonspecific dehydrogenase enzyme (with anaerobic incubation only). *Campylobacter* strains (including WAL 10732/FDC 286) had different MDH-specific bands of variable migration distances, both under aerobic and anaerobic incubation conditions.

Cellular fatty acid analysis The fatty acid methyl esters (FAME) found in the different groups of organisms are listed in Table BXII.β.40. Cluster analysis of the cellular fatty acid data indicated three major groups: (1) typical *C. gracilis* (including the strain ATCC 33236^T, (2) the *Sutterella* group, and (3) *Campylobacter* species. Among other differences, the absence of $C_{12:0}$ fatty-acid-methyl-ester (FAME) distinguished *Sutterella* from typical *C. gracilis* and other *Campylobacter* species. The typical *C. gracilis, Campylobacter,* and *B. ureolyticus* also cluster quite independently of each other. The strain chosen as the type strain of *Sutterella* (WAL 9799) was selected because it resided in the center of the *Sutterella* cluster. *C. gracilis* ATCC strain 33236^T clustered at the edge of the cellular fatty acid cluster analysis of *C. gracilis.*

16S rRNA sequencing Phylogenetic relationships of *Sutterella* are shown in the chapter describing the family *Alcaligenaceae* (Fig. BXII.β.21).

Sequencing of the 16S rRNA gene was performed for two *Sutterella* strains (WAL 9054 [GenBank L37786] and WAL 7877 [GenBank L37785], *Campylobacter gracilis* strain ATCC 33236^T (GenBank Collection L37787), and *C. rectus* WAL 7943. The 16S rRNA sequences of the two *Sutterella* strains (WAL 9054 and WAL 7877 [1454 and 1419 base pairs sequenced, respectively]) were identical to each other but did not cluster with either the *C. gracilis* or *Campylobacter* strains analyzed or those contained in the databases GenBank or RDP. *C. gracilis* strain ATCC 33236^T branches at the same point as *Campylobacter rectus.*

DNA hybridization The *Sutterella wadsworthensis* type strain did not hybridize with DNA from either *C. gracilis* (10 strains) or other *Campylobacter* species (9 strains), but hybridized strongly (>96%) with DNA from 68% of the *Sutterella* (19 strains) and exhibited hybridization values of >67% with all of the strains tested, indicating that these strains are of the same species or very closely related species that cannot be differentiated phenotypically. Ten *Sutterella* strains did not hybridize at all with the *C. gracilis* type strain, and the DNA of three *Sutterella* strains hybridized very slightly (3%, 5%, and 12%); seven strains of *Campylobacter* did not hybridize at all with *C. gracilis* ATCC 33236^T, and two strains of *C. rectus* hybridized slightly (15%). *C. gracilis* strain ATCC 33236^T hybridized moderately with other typical *C. gracilis* strains (2/9 strains hybridized at 70–80%, 3/9 at 40–50%, 3/9 at 30–50%, and 1/9 at 22%). *C. gracilis* strain WAL 8030 hybridized more strongly with other *C. gracilis* strains, did not hybridize at all with *Sutterella,* and hybridized slightly (23%) with the one *C. rectus* strain tested.

Clinical source The strains belonging to the *Sutterella* group were isolated mainly from infections of gastrointestinal origin (Finegold and Jousimies-Somer, 1997; Jousimies-Somer, 1997). Strains belonging to *C. gracilis* were found virtually only in infections above the diaphragm (e.g., brain, pleural fluid, etc.); only one strain of *C. gracilis* was found from an appendiceal specimen. The resistance of *Sutterella* to both human bile (unpublished data from our laboratory) and oxgall is notable; this may account for their ability to survive in the biliary tract and bowel.

Antimicrobial susceptibility Over 95% of *Sutterella* strains are susceptible to amoxicillin/clavulanate, ticarcillin/clavulanate, cefoxitin, ceftriaxone, and clindamycin. 85–95% of strains are susceptible to piperacillin, piperacillin/tazobactam, ceftizoxime, ciprofloxacin, trovafloxacin, azithromycin, clarithromycin, erythromycin, and roxithromycin.

ENRICHMENT AND ISOLATION PROCEDURES

Sutterella wadsworthensis is isolated on *Brucella* blood agar with 5% lysed sheep blood, 1 μg/ml Vitamin K_1, 1 μg/ml hemin, 1% formate/fumarate (used as a growth supplement). Colonies are seen after 48 h at 37°C in an anaerobic chamber. Colonies are circular, entire, convex, yellow to brown, translucent to opaque, 1–1.5 mm in diameter.

MAINTENANCE PROCEDURES

Strains are kept in skim milk (20%) at −70°C.

DIFFERENTIATION OF THE GENUS *SUTTERELLA* FROM OTHER GENERA

Sutterella can be differentiated from *Campylobacter gracilis* and from other *Campylobacter* species as shown in Table BXII.β.39.

Dehydrogenase patterns are distinctive from those of *C. gracilis* and other campylobacters when the gels are incubated anaerobically. Cellular fatty acid analysis indicates that the cluster of *Sutterella* is distinct from the (separate) clusters formed by *C. gracilis*, *Bacteroides ureolyticus*, or other *Campylobacter* species. 16S rRNA sequence and DNA hybridization data indicate that *Sutterella* is distinct from *C. gracilis* and other *Campylobacter* species at the genus and species levels.

TAXONOMIC COMMENTS

Historical notes During biochemical characterization and susceptibility testing of isolates from clinical specimens in the Wadsworth Anaerobe laboratory, we found heterogeneity among the strains previously identified as *Campylobacter gracilis*. These organisms differed with regard to their bile resistance and ability to reduce triphenyltetrazolium chloride (TTC). Isolates that were bile resistant and unable to reduce TTC aerobically were compared to other strains of *C. gracilis* (including the ATCC type strain) and five species of *Campylobacter* using biochemical characteristics, cellular fatty acid profiles, DNA relatedness, and 16S rRNA sequence homology.

Campylobacter gracilis was originally known as *Bacteroides gracilis*, one of the members of the *B. ureolyticus* group. These are asaccharolytic, nitrate-positive organisms that require formate or hydrogen as an electron donor and may pit the agar. *B. ureolyticus* and *B. gracilis* clearly do not belong in *Bacteroides sensu stricto* (currently limited to the *B. fragilis* group; Shah and Collins, 1989), and 16S rRNA sequence analysis and DNA–rRNA hybridization studies support description of a tight homology group that includes *B. ureolyticus*, *B. gracilis*, and *Campylobacter* (including some previous *Wolinella* species) (Paster and Dewhirst, 1988; Whiley and Beighton, 1991). Both *B. ureolyticus* and *B. gracilis* are microaerophiles and not anaerobes; it has been proposed that both be included in the group of "true campylobacters" (Han et al., 1991). *B. gracilis* was reclassified as *Campylobacter gracilis*; *B. ureolyticus* was not renamed along with *C. gracilis*; its recommended status was a *species incertae sedis* pending further investigation (Vandamme et al., 1995a).

Strains belonging to *C. gracilis* were (with the exception of one strain) isolated from infections above the diaphragm, while the *Sutterella* strains were mostly isolated from infections below the diaphragm; the metronidazole-resistant strains all belonged to *Sutterella* (Molitoris et al., 1997).

List of species of the genus Sutterella

1. **Sutterella wadsworthensis** Wexler, Reeves, Summanen, Molitoris, McTeague, Duncan, Wilson and Finegold 1996a, 257[VP]

wads.worth'en.sis. M.L. adj. *wadsworthensis* from Wadsworth,

referring to the Wadsworth Anaerobe Laboratories, VAMC, West Los Angeles, where the strains were identified.

The characteristics are as described for the genus and as shown in Tables BXII.β.39 and BXII.β.40.

TABLE BXII.β.39. Differentiation of *Sutterella wadsworthensis* from related taxa

Characteristic	*S. wadsworthensis*	*B. ureolyticus*	*Campylobacter*	*Campylobacter gracilis*
Oxidase	−	+	+	−
Urease	−	+	−	−
Indoxyl acetate	−	NT[a]	Var[b]	+
Growth in 2% O_2	+	+	+	+
Growth in 6% O_2	+ (−)	+ or w	+	−[c]
Resistance to 20% bile	R	S	Var[d]	S
TTC reduction[e]	−	NT	+	+

[a]NT, not tested.

[b]Variable, even within the same species.

[c]*C. gracilis* only grew in 6% oxygen on plates containing *Brucella* agar with formate/fumarate but no blood added; if blood was added, *C. gracilis* did not grow.

[d]Strains tested included *C. curvus* (Res), 2; *C. rectus* (Sens), 4; *C. concisus* (Sens), 1; *Campylobacter* sp. 1 (Strain WAL 4864), Intermediate.

[e]Ability to reduce TTC in an aerobic atmosphere when formate/fumarate is added to the media.

TABLE BXII.β.40. Cellular fatty acid methyl esters of *Sutterella* and related taxa

Characteristic	*S. wadsworthensis* (30 strains)	*Campylobacter gracilis* (formerly *B. gracilis*) (10 strains)	*Campylobacter* (16 strains)[a]
$C_{12:0}$ FAME[b]	<1	10–20	4–13
$C_{14:0}$ FAME	3–8.5	8–13	8.5–13
$C_{16:1\ \omega7c}$ FAME	17–24	<5	9–18
$C_{16:0}$ FAME	26–44	6–12	22–34
$C_{16:0}$ DMA[c]	0.2–6	15–23	2–5[d]
$C_{18:1\ \omega9c}$ DMA[c]	0–1	10–12	0
Summed feature 10[e]	11–30	10–20	16–30

[a] *C. rectus*, 8 strains; *C. curvus*, 3 strains; *C. concisus* 3 strains; *Campylobacter* sp., 2 strains.

[b]Fatty acid methyl ester.

[c]DMA, dimethyl acetal.

[d] *C. curvus*, 2–5%; other *Campylobacter* strains, 0.

[e]18:1 C11/t9/t6 FAME and/or unknown peak at 17.834.

The mol% G + C of the DNA is: unknown.
Type strain: Strain WAL 9799, ATCC 51579.
Additional Remarks: The type strain was selected because

it resided in the center of the *Sutterella* cluster in the cellular fatty acid analysis.

Genus IX. **Taylorella** Sugimoto, Isayama, Sakazaki and Kuramochi 1984, 503[VP] (Effective publication: Sugimoto, Isayama, Sakazaki and Kuramochi 1983, 155)

Nancy M.C. Bleumink-Pluym and Bernard A.M. van der Zeijst

Tay' lor.el.la. M.L. dim. ending *-ella* M.L. fem. n. *Taylorella* named after C.E.D. Taylor, who first studied the organism.

Generic definition: **Short rods**, $0.7 \times 0.7–1.8\,\mu m$. **Gram negative**. Nonmotile. Requires an atmosphere enriched with **5–10% CO_2.** Growth on rich peptone based chocolate agar medium at 35–37°C. Colonies are circular, smooth, grayish-white and 0.5–3.0 mm in diameter after an incubation period of 3–6 d. **Oxidase positive.** Catalase, phosphatase, and phosphoamidase are produced. Acid is not produced from carbohydrates. The phylogenetic position based on 16S rDNA analysis is in the *Betaproteobacteria*. The 16S rRNA contains the signature sequences of the *Betaproteobacteria* in 63 of 66 positions.

The mol% G + C of the DNA is: 36.5.

Type species: **Taylorella equigenitalis** (Taylor, Rosenthal, Brown, Lapage, Hill and Legros 1978) Sugimoto, Isayama, Sakazaki and Kuramochi 1984, 503 (Effective publication: Sugimoto, Isayama, Sakazaki and Kuramochi 1983, 155) (*Haemophilus equigenitalis* Taylor, Rosenthal, Brown, Lapage, Hill and Legros 1978, 136.)

FURTHER DESCRIPTIVE INFORMATION

The initial name of this bacterium was *Haemophilus equigenitalis* (Taylor et al., 1978). It was classified based on its mol% G + C content in the family *Pasteurellaceae* as a *species incertae sedis* belonging to the genus *Haemophilus*. In 1983, based on DNA hybridization data, Sugimoto proposed the new genus *Taylorella* with *Taylorella equigenitalis* as the only species (Sugimoto et al., 1983). This was validated in the IJSB in 1984. The 16S rDNA sequence of *Taylorella equigenitalis* was determined in 1993 (Bleumink-Pluym et al., 1993) and a phylogenetic analysis of this sequence revealed a position in the *Betaproteobacteria* apart from the position of *Haemophilus influenzae*, which belongs to the *Gammaproteobacteria*.

Cells of *Taylorella equigenitalis* are short rods or usually coccobacilli. The outer membrane can be covered by a layer of capsular-like material (Hitchcock et al., 1985; Bleumink-Pluym, 1995). Fimbriae have only been demonstrated *in vivo* (Kanemaru et al., 1992). The most abundant outer membrane protein has a molecular mass of 39–41 kDa (Sugimoto et al., 1988). The N-terminus of this immunodominant OMP is similar to that of porins from *Bordetella pertussis*, *Neisseria gonorrhoeae*, and *Neisseria meningitidis* (Bleumink-Pluym, 1995).

T. equigenitalis grows well on agar medium containing a rich peptone base with 5% chocolated sheep blood. Horse blood is not recommended because of the possible presence of antibodies directed toward *T. equigenitalis*. The medium should be supplemented with sodium sulfite (200 mg/l) and L-cysteine hydrochloride (100 mg/l). The plates should be incubated in an atmosphere enriched with 5–10% CO_2. Growth is observed over a temperature range of 30–41°C; optimal temperature is 37°C.

Visible colony formation requires at least 48 h. Colonies are circular, smooth, raised, and shiny. They vary in size from 0.5–3.0 mm, are grayish-white, and can subsequently turn yellowish-brown. Sometimes the colonies can be moved freely over the surface of the agar.

Acid is not produced from carbohydrates. Oxidase, catalase, phosphatase, and phosphoamidase are produced. Nitrates and nitrites are not reduced. Lysine and ornithine decarboxylase, arginine dihydrolase, gelatinase, lipase, urease, and deoxyribonuclease are not produced. Indole and H_2S not produced (Sugimoto et al., 1983).

The 16S rDNA sequence of *T. equigenitalis* was compared with entries present in the EMBL and GenBank database. Close relationships with 16S rDNA sequences of *Alcaligenes xylosoxidans* (94.2%) and *Bordetella bronchiseptica* (93.5%) were detected. Two variable regions that distinguish the three closest relatives most effectively are located at the 5′ end of the 16S rDNA at positions 65–97 and 412–478 (*E. coli* numbering).

Hybridizations between DNAs from two different *Taylorella* strains and DNAs from strains of *Bordetella*, *Moraxella*, *Kingella*, *Legionella*, *Haemophilus*, and *Brucella* species revealed no significant relatedness. Relatedness of DNAs from five *Taylorella* strains showed reassociation values of about 68% and a thermal stability difference of 2.5% (Sugimoto et al., 1983), essentially fulfilling the genetic definition of a species.

The genome size of *T. equigenitalis* is 1.5×10^6 bp. DNA of different isolates can be cleaved in a few large fragments by the restriction endonuclease *ApaI*, which recognizes the infrequently occurring GGGCCC sequence. Different genomic restriction patterns can be observed by using field inversion gel electrophoresis (FIGE) (Bleumink-Pluym et al., 1990; Engval et al., 1991; Matsuda et al., 1993). *T. equigenitalis* strains from different outbreaks generally have different genomic restriction patterns.

By immunoblotting, Sugimoto et al. demonstrated that mares experimentally infected with *T. equigenitalis* developed antibodies to a 41-kDa major outer membrane protein (Sugimoto et al., 1988). The N-terminus of this immunodominant OMP is similar to that of porins from *Bordetella pertussis*, *Neisseria gonorrhoeae*, and *Neisseria meningitidis* (Bleumink-Pluym, 1995).

T. equigenitalis is moderately resistant to clindamycin, lincomycin, trimethoprim, and sulfamethoxazole (MIC 32 µg/ml); sensitive to penicillin G, ampicillin, carbenicillin, cephaloridine, cephalotin, erythromycin, tetracycline, kanamycin, gentamicin, neomycin, chloramphenicol, and polymyxin B (Sugimoto et al., 1983). The majority of *T. equigenitalis* strains isolated internationally are resistant to streptomycin (MIC >512), but streptomycin-susceptible strains exist.

Clinical signs of *T. equigenitalis* infection in horses have been reported only in the mare and can be vaginal discharge, infertility, or early abortion. In the acute state of infection a widespread endometritis with local erosion of surface epithelium can be observed. The effect of the disease on fertility can be attributed to acute endometrial inflammation. The principal lesions are in the uterus and are characterized by destruction of endometrial epithelial cells and infiltration of neutrophils into the epithelium and lamina propria of the uterus. Histopathological studies have demonstrated that *T. equigenitalis* adheres to the cilia of the epithelial cells and proliferates on the endometrium (Wada et al., 1983). *In vitro, T. equigenitalis* adheres to and invades equine dermal cells. These abilities differ between strains and do not correlate with their genomic restriction patterns (Bleumink-Pluym et al., 1996).

The principal sites of colonization in mares are the urogenital membranes of the clitoral sinuses and fossa, urethra, and cervix. In stallions, the principal sites of colonization are the urogenital membranes of the urethra, urethral fossa, and penile sheath. *Taylorella* can be regarded as a commensal or opportunist as the bacterium is mostly isolated from asymptomatic carriers. Likewise, the prevalence of *Taylorella* in the horse population is high as demonstrated by PCR assay (Bleumink-Pluym et al., 1994).

ENRICHMENT AND ISOLATION PROCEDURES

Taylorella requires rather specific growth conditions and is rapidly overgrown by other bacteria resident in the genital tract of horses. Genital swabs are inoculated on Colombia blood agar base (Oxoid Ltd)-chocolated agar supplemented with sodium sulfite (200 mg/l) and L-cysteine hydrochloride (100 mg/l) (Atherton, 1983) with and without the addition of 5 μg of clindamycin, 1 μg trimethoprim, and 5 μg of amphotericin-B per ml. Plates are incubated with 7% CO_2 in air at 37°C for 6 d. Colonies are circular, smooth, grayish-white, and vary in size from 0.5–3.0 mm. Colonies suspected of being *T. equigenitalis* are Gram stained and tested for oxidase. Slide agglutination, indirect immunofluorescence with hyperimmune serum, or PCR detection based on 16S rDNA sequences can be used for confirmation.

MAINTENANCE PROCEDURES

Plate cultures will survive for 3 weeks without subcultivation but should be kept in an atmosphere enriched with 5–10% CO_2. All strains can be maintained for up to 4 yr at −70°C in brain heart infusion broth containing 15% glycerol. Lyophilization, e.g., in skim milk, can also be used for conserving cultures.

DIFFERENTIATION OF THE GENUS *TAYLORELLA* FROM OTHER GENERA

The differential characteristics of the genus *Taylorella* are listed in Table BXII.β.41.

TAXONOMIC COMMENTS

The phylogenetic position of *Taylorella equigenitalis* in the *Betaproteobacteria* differs from that of *Haemophilus influenzae*, which belongs to the *Gammaproteobacteria*, and supports its exclusion from the family *Pasteurellaceae*. *Taylorella* does not seem to fit in one of the known bacterial families. Based on 16S rDNA analysis *Taylorella* is most closely related to *Bordetella* and *Alcaligenes*. Although these species share substantial 16S rDNA sequences (93.5% and 94.2%, respectively), it is not likely that they belong to the same family. No relatedness was detected in a DNA–DNA hybridization between *Taylorella* and *Bordetella*, which is in accord with the variation in the mol% G + C contents of their genomes. *Bordetella* and *Alcaligenes* have a mol% G + C content of ~69, while *Taylorella* has a mol% G + C content of 36.5. Members of the genera *Bordetella*, *Alcaligenes*, and *Taylorella* occur in different ecological niches. The principal habitat of *Taylorella* is the mucosal membrane surface of the genital tract of horses. Certain phenotypic characteristics are shared with other bacteria harboring mucous membranes of the genital tract, like *Neisseria gonorrhoeae*. Both species are transmitted sexually, cause similar clinical signs of infection (*Taylorella* in horses; *N. gonorrhoeae* in humans), have similar culture conditions, are oxidase positive, have variable colony morphology, and have a similarity in the N-terminal amino acid sequences of the 41-kDa and 17-kDa outer membrane protein.

List of species of the genus Taylorella

1. **Taylorella equigenitalis** (Taylor, Rosenthal, Brown, Lapage, Hill and Legros 1978) Sugimoto, Isayama, Sakazaki and Kuramochi 1984, 503[VP] (Effective publication: Sugimoto, Isayama, Sakazaki and Kuramochi 1983, 155) (*Haemophilus equigenitalis* Taylor, Rosenthal, Brown, Lapage, Hill and Legros 1978, 136.)

   e.qui.ge.ni.ta′lis. L. gen. n. *equi* of the horse; L. adj. *genitalis* genital; L. adj. *equigenitalis* genital of the horse.

   All characteristics are as described for the genus *Taylorella*.

   This strain was isolated in 1977 from a cervical swab of a thoroughbred mare with contagious metritis.

   The mol% G + C of the DNA is: 36.5 (T_m).

   Type strain: Strain 61717/77, CIP 7909, DSM 10668, NCTC 11184.

   GenBank accession number (16S rRNA): X68645.

TABLE BXII.β.41. Differentiation of the genus *Taylorella* from other closely related taxa[a]

Characteristic	*T. equigenitalis*	*Bordetella avium*	*Bordetella bronchiseptica*	*Bordetella holmesii*	*Bordetella parapertussis*	*Bordetella pertussis*	*Alcaligenes faecalis*	*Alcaligenes xylosoxidans*
Strictly aerobic	−	+	+	+	+	+	+	+
Urease	−	−	+	−	+	−	−	−
Oxidase	+	+	+	−	−	+	+	+
Motility	−	+	+	−	−	−	+	+
Mol% G + C	36.5	61.6	68.9	61.9	66.7	67.7	56	69

[a]For symbols see standard definitions.

Family IV. Comamonadaceae Willems, De Ley, Gillis and Kersters 1991a, 447[VP]

ANNE WILLEMS AND MONIQUE GILLIS

Co.ma.mo.na.da' ce.ae. M.L. fem. n. *Comamonas* type genus of the family; suff. *-aceae* to denote a family; M.L. fem. pl. n. *Comamonadaceae* the *Comamonas* family.

Cells are **straight** or **slightly curved rods or spirilla**. Gram negative. Most genera are **motile by a single polar flagellum or by bipolar tufts of 1–5 flagella**; one genus (*Variovorax*) is motile by sparse peritrichous flagella, and another (*Brachymonas*) lacks flagella. No endospores are formed. Coccoid bodies are rarely formed, but do occur in some (*Aquaspirillum*) species. (The use of square brackets indicates these species are not true aquaspirilla, which are related to type species *Aquaspirillum serpens*.) **Chemoorganotrophic or facultatively chemolithotrophic with H_2 or CO oxidation.** Possess a strictly respiratory type of metabolism, with oxygen as the terminal electron acceptor. Some species can also use nitrates. **Oxidase positive**, except for the genus *Xylophilus*. **Optimal growth temperature ranges from 24 to 35°C**, except for *Polaromonas* and [*Aquaspirillum*] *psychrophilum*, which prefer 4 and 18°C, respectively. Yellow, insoluble pigments are produced by the genera *Hydrogenophaga*, *Variovorax*, and *Xylophilus*. Nitrogen fixation has been reported for some *Hydrogenophaga* species. A wide variety of organic acids, including amino acids, is used, but sugars are rarely attacked. **Major fatty acids are palmitoleic acid ($C_{16:1}$), palmitic acid ($C_{16:0}$) and *cis*-vaccenic acid ($C_{18:1\ \omega7c}$)**, and the major quinone is **ubiquinone Q-8**.

Members of the *Comamonadaceae* have been **isolated from soil, mud, and water, in natural and industrial environments**. Strains from the genera *Comamonas* and *Acidovorax* have also been isolated from clinical samples, but are not regarded as pathogenic. The genus *Xylophilus* is pathogenic to grapevines and some members of the genus *Acidovorax* are pathogenic to grasses, orchids, and members of the cucumber family.

The *Comamonadaceae* belong to the *Betaproteobacteria*. At present the following genera belong to this family, on phylogenetic grounds: *Comamonas*, *Acidovorax*, *Alicycliphilus*, *Brachymonas*, *Caldimonas*, *Delftia*, *Diaphorobacter*, *Hydrogenophaga*, *Lampropedia*, *Macromonas*, *Polaromonas*, *Ramlibacter*, *Rhodoferax*, *Variovorax*, *Xenophilus*, and *Xylophilus*. In addition, a number of misnamed *Aquaspirillum* species belong to the *Comamonadaceae* on phylogenetic grounds. The genera *Aquabacterium*, *Ideonella*, *Leptothrix*, *Roseateles*, *Rubrivivax*, *Schlegelella*, *Sphaerotilus*, *Tepidimonas*, *Thiomonas*, and *Xylophilus* have been placed in the *Comamonadaceae* as *genera incertae sedis*. The genera *Alicycliphilus*, *Caldimonas*, *Diaphorobacter*, *Ramlibacter*, and *Schlegelella* were described after the cut-off date for inclusion in this volume. Differentiating features for a number of these taxa are presented in Table BXII.β.42.

The mol% G + C of the DNA is: 52–70.

Type genus: **Comamonas** De Vos, Kersters, Falsen, Pot, Gillis, Segers and De Ley 1985b, 450 emend. Tamaoka, Ha and Komagata 1987, 57; emend. Willems, Pot, Falsen, Vandamme, Gillis, Kersters and De Ley 1991c, 438.

TAXONOMIC COMMENTS

The family *Comamonadaceae* has been proposed as a formal taxon for the so-called acidovorans rRNA complex (Willems et al., 1991a). This was described as one of the rRNA groups within the large genus *Pseudomonas* in the previous edition of this *Manual* (Palleroni, 1984). Previously, it had been apparent from extensive phenotypic studies that this genus contained several species groups (Stanier et al., 1966). The genotypic basis for these groupings was revealed when the genus *Pseudomonas* was shown by DNA–rRNA hybridizations to consist of at least five main rRNA groups (Palleroni et al., 1973), and further DNA–rRNA hybridizations have shown these groups to be only remotely related to each other (De Vos and De Ley, 1983). The same groupings also have been supported by serological data (Baumann and Baumann, 1978), regulatory patterns in the biosynthesis of aromatic amino acids (Byng et al., 1983), coliphage QB host factor activity and antigenicity (Dubow and Ryan, 1977), fatty acid composition (Oyaizu and Komagata, 1983), and 16S rRNA oligonucleotide cataloging (Woese et al., 1984a).

In addition to at least 10 different *Pseudomonas* species, the *Pseudomonas acidovorans* rRNA group also contained *Comamonas terrigena*, *Xanthomonas ampelina*, *Alcaligenes paradoxus*, and several *Aquaspirillum* species (De Vos and De Ley, 1983; De Vos et al., 1985a, b; Willems et al., 1991a). It thus included common water and soil inhabitants, clinical isolates, plant pathogens, and hydrogen and CO oxidizers. Except for *Comamonas terrigena*, all of these species were phylogenetically unrelated to the type species of their respective genera, and in a series of polyphasic studies, they were therefore gradually transferred to other new or existing genera. An overview of these changes is presented in Table BXII.β.43. Since the original proposal of the family *Comamonadaceae*, four new genera (*Brachymonas*, *Delftia*, *Polaromonas*, and *Rhodoferax*) have been proposed within this group and these are listed in Table BXII.β.43. Not included is *Xenophilus*, which was described after the completion of this chapter (Blümel et al., 2001a).

This subdivision of the *Comamonadaceae* into different genera has relied heavily on DNA–rRNA hybridization data and has now been confirmed and extended by rRNA gene sequence analysis (Wen et al., 1999). Most members of the *Comamonadaceae* share at least 94–95% rDNA sequence similarity. A dendrogram showing the phylogenetic relationships of the *Comamonadaceae* and their nearest neighbors is shown in Fig. BXII.β.27. It was calculated using 16S rRNA gene sequences available from the EMBL database. From these data, it is clear that the genera *Acidovorax*, *Hydrogenophaga*, *Delftia*, *Polaromonas*, *Brachymonas*, and *Rhodoferax* and the species [*Aquaspirillum*] *gracile* and [*Aquaspirillum*] *sinuosum* are well separated. It is also apparent that the relationships between *Xylophilus* and *Variovorax*, and between [*Aquaspirillum*] *metamorphum* and [*Aquaspirillum*] *psychrophilum* need further study. The bootstrap value for the grouping of *Comamonas terrigena* and *Comamonas testosteroni* is relatively low (70; Fig. BXII.β.27), indicating that this is not a very robust grouping. It may be rearranged as more sequences are included.

It seems quite likely that additional genera will be included in this family, not only because of reclassification of some of the above listed species and genera, but also because of newly isolated groups. Many of the members of the *Comamonadaceae* are common inhabitants of water, soil, and polluted environments, and are regularly isolated in ecological studies. Some *Comamonadaceae*

TABLE BXII.β.42. Differentiating features for the members of the family *Comamonadaceae* [a]

Characteristic	*Comamonas*	*Acidovorax*	*Brachymonas*	*Delftia*	*Hydrogenophaga*	*Polaromonas*	*Rhodoferax*	*Variovorax*	*Xylophilus*	*[Aquaspirillum]* [b]
Cell morphology:										
Rods		+		+	+	+		+	+	
Curved rods							+			
Rods to spirilla	+									
Cocci to short rods			+							
Spirilla										+
Gas vacuoles	−	−	−	−	−	+	−	−	−	−
Flagella:										
Number	1–5	1		1–5	1–2	1	1		1	
Location:										
Polar	+	+			+	+	+		+	+[c]
Polar tufts	+			+						
Bipolar tufts				+						+
Subpolar						+				
Peritrichous								+		
Isolated from:										
Soil	+	+	−	+	+	−	−	+	−	−
Fresh water	+	+	−	+	+	−	−	+	−	+
Seawater	−	−	−	−	−	+	−	−	−	−
Activated sludge	+	+	+	+	−	−	+	−	−	−
Plant tissue	−	+	−	−	−	−	−	−	+	−
Clinical samples	+	+	−	+	−	−	−	−	−	−
Optimal growth temperature, °C	30	30–35	30–35	30	30–35	4	25–30	30	24	30–32[d]
Growth at 37°C	+	d	+	d	d	−	−	nd	−	D
Growth on nutrient agar	+	+	+	+	+	+	+	+	+	−
Yellow pigment	−	−	−	−	+	−	−	+	+	−
Growth with 3% NaCl	d	d	+	d	nd	+	−	nd	−	−
Growth on glucose	−	D[e]	−	−	+	d	+	+	d	D[f]
Growth factors[g]	M[h] N	nd	−	−	−	aa	B, T	nd	G	−
Autotrophic growth with H$_2$	−	d	−	−	+	−	nd	d	−	−
Phototrophic growth	−	−	−	−	−	−	+	−	−	−
Anaerobic growth with nitrate as terminal electron acceptor	−	d	+	−	D	−	nd	−	−	D[i]
Denitrification	−	d	+	−	D	−	nd	−	−	D[i]
Major quinones: [j]										
Q-8	+	+	+	+	+		+	+		
RQ-8			+				+			
Major 3-OH fatty acids:										
C$_{8:0}$				+	+		+			
C$_{10:0}$	+	+	+	+				+		
Mol% G + C of the DNA	60–69	62–70	63–65	67–90	65–69	52–57	60	67–69	68–69	57–59

[a]For symbols see standard definitions; nd, not determined.

[b]In this table the *[Aquaspirillum]* species are listed grouped in a single column, but they are likely to represent several additional genera (Wen et al., 1999).

[c] *[Aquaspirillum] delicatum* has 1 or 2 flagella at one pole (Krieg, 1984b).

[d]The optimal temperature for *[Aquaspirillum] psychrophilum* is 20°C (Krieg, 1984b).

[e]Only *A. konjaci* does not use glucose (Willems et al., 1992a).

[f]Glucose is used only by *[Aquaspirillum] gracile* (Krieg, 1984b).

[g]M, methionine; N, nictotinamide; aa, amino acids; B, biotin; T, thiamine; G, glutamic acid.

[h]Methionine and nicotinamide are required only by *C. terrigena* and the former *[Aquaspirillum] aquaticum* requires niacin (Pot et al., 1992b).

[i]Denitrification and anaerobic growth with nitrate is positive for *[Aquaspirillum] psychrophilum* (Krieg, 1984b).

[j]R, ubiquinone; RQ, rhodoquinone.

have the capacity to degrade complex organic compounds and are of potential use in degradation applications and bioremediation, topics which are the focus of intense research and which result in many new isolates. Some of these new strains may belong to new groups within the *Comamonadaceae*, as has occurred with *Rhodoferax* (Hiraishi, 1994), *Brachymonas* (Hiraishi et al., 1995b) and *Polaromonas* (Irgens et al., 1996).

As mentioned above, the family *Comamonadaceae* encompasses a remarkable diversity of phenotypic traits. In the past, different properties have often been studied in members of this family belonging to different phenotypic groups. This makes phenotypic comparison between members difficult and often incomplete. The accumulation of more 16S rDNA and other sequence data will become increasingly important for the classification within this group. As was shown previously (Amann et al., 1996), it may be possible to derive oligonucleotide probes for the identification of the various genera. The restriction analysis of amplified rRNA genes of a limited number of *Comamonadaceae* has

TABLE BXII.β.43. Members of the family *Comamonadaceae*

Current name[a]	Subjective synonym(s)	Reference
Acidovorax delafieldii	*Pseudomonas delafieldii*, EF group 13	Willems et al., 1990
Acidovorax facilis	*Pseudomonas facilis*	Willems et al., 1990
Acidovorax temperans	EF group 16	Willems et al., 1990
Acidovorax avenae subsp. *avenae*	*Pseudomonas avenae*, *Pseudomonas rubrilineans*, "*Pseudomonas setariae*"	Willems et al., 1992a
Acidovorax avenae subsp. *citrulli*	*Pseudomonas pseudoalcaligenes* subsp. *citrulli*	Willems et al., 1992a
Acidovorax konjaci	*Pseudomonas pseudoalcaligenes* subsp. *konjaci*	Willems et al., 1992a
Brachymonas denitrificans	new	Hiraishi et al., 1995b
Comamonas terrigena	"*Comamonas terrigena*", *Aquaspirillum aquaticum*, EF group 10	De Vos et al., 1985b, Willems et al., 1991c
Comamonas testosteroni	*Pseudomonas testosteroni*	Tamaoka et al.,1987
Delftia acidovorans	*Comamonas acidovorans*, *Pseudomonas acidovorans*	Wen et al., 1999
Hydrogenophaga flava	*Pseudomonas flava*	Willems et al., 1989
Hydrogenophaga palleronii	*Pseudomonas palleronii*	Willems et al., 1989
Hydrogenophaga pseudoflava	*Pseudomonas pseudoflava*	Willems et al., 1989
Hydrogenophaga taeniospiralis	*Pseudomonas taeniospiralis*	Willems et al., 1989
Polaromonas vacuolata	new	Irgens et al., 1996
Rhodoferax fermentans	new	Hiraishi et al., 1991a
Variovorax paradoxus	*Alcaligenes paradoxus*	Willems et al., 1991a
Xylophilus azovorans	new	Blümel et al., 2001a
Xylophilus ampelinus	*Xanthomonas ampelina*	Willems et al., 1987
[Aquaspirillum] anulus		Willems et al., 1991c
[Aquaspirillum] delicatum		Willems et al., 1991c
[Aquaspirillum] giesbergeri		Willems et al., 1991c
[Aquaspirillum] gracile		Willems et al., 1991c
[Aquaspirillum] metamorphum		Willems et al., 1991c
[Aquaspirillum] psychrophilum		Willems et al., 1991c
[Aquaspirillum] sinuosum		Willems et al., 1991c

[a]Square brackets are used to indicate that a genus allocation is phylogenetically unsound because the species is phylogenetically unrelated to its type species.
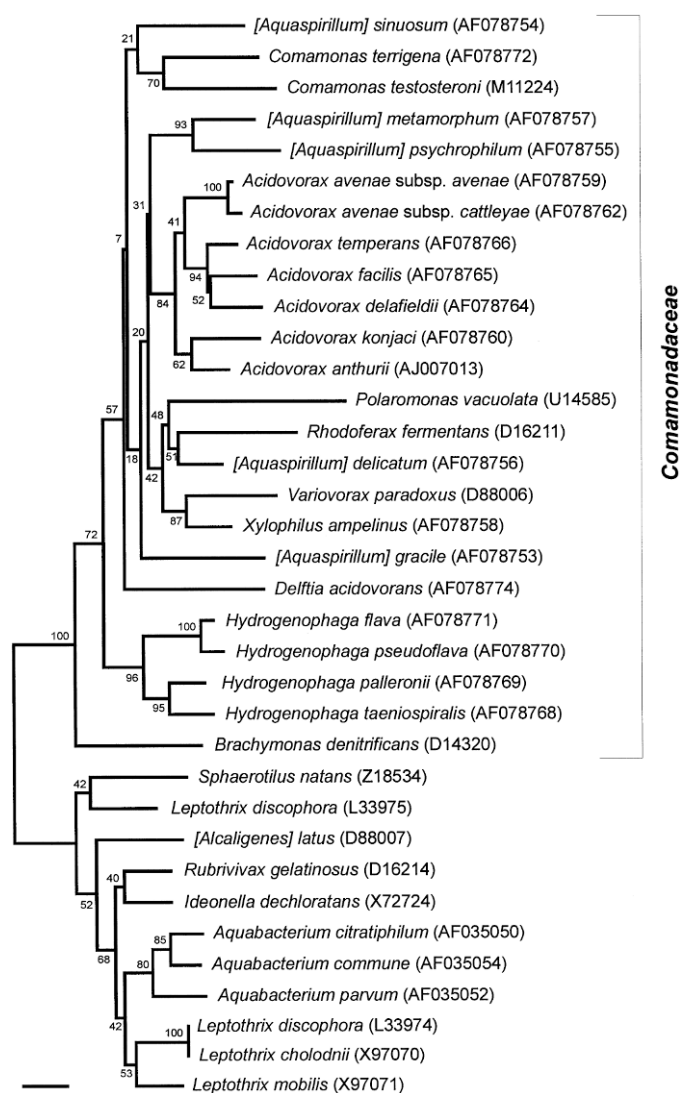
FIGURE BXII.β.27. Dendrogram showing the phylogenetic relationships of the *Comamonadaceae* and their closest relatives. The dendrogram is based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library (accession numbers are given in brackets) and was constructed after multiple alignment of data, calculation of distances (corrected according to the Kimura-2 model, using a stretch of 1360 aligned positions) and clustering with the neighbor-joining method. Bootstrap values based on 500 replications are listed at the branching points. Programs from the GCG and PHYLIP packages (Felsenstein, 1982; Devereux et al., 1984) were used on the BEN computer facility operated at the Brussels Free University Computing Centre. Bar = 1 estimated substitution per 100 bases.

been shown to allow differentiation of species, but not always genera (Vaneechoutte et al., 1992).

The nearest phylogenetic neighbors of the *Comamonadaceae* are the photosynthetic genus *Rubrivivax*, the sheath-forming *Leptothrix* and *Sphaerotilus*, the hydrogen oxidizers *[Alcaligenes] latus* and *[Pseudomonas] saccharophila* (Willems et al., 1991b), *Ideonella* (Malmqvist et al., 1994a), and *Aquabacterium* (Kalmbach et al., 1999).

Genus I. **Comamonas** *De Vos, Kersters, Falsen, Pot, Gillis, Segers and De Ley 1985b,*
450^{VP} emend. Tamaoka, Ha and Komagata 1987, 57, emend. Willems, Pot, Falsen,
Vandamme, Gillis, Kersters and De Ley 1991c, 438

ANNE WILLEMS AND MONIQUE GILLIS

Co.ma.mo′ nas. L. n. *coma* lock of hair; Gr. n. *monas* a unit, monad; M.L. fem. n. *Comamonas* cell with a polar tuft of flagella.

Straight or slightly curved rods or spirilla, 0.3–0.8 × 1.1–4.4 μm; occasionally longer (5–7 μm), irregularly curved cells or spirilla may occur. Cells occur separately or in pairs and **are motile by means of polar or bipolar tufts of 1–5 flagella** except for *C. koreensis*, which is nonmotile. **Gram negative.** No diffusible pigments are produced on nutrient agar. **Oxidase and catalase positive. Aerobic. Chemoorganotrophic**, oxidative carbohydrate metabolism with oxygen as the terminal electron acceptor. *C. nitrativorans* is also capable of denitrification. Good growth on media containing organic acids, amino acids, or peptone; few carbohydrates are used. Major fatty acids are hexadecanoic acid ($C_{16:0}$), hexadecenoic acid ($C_{16:1}$) and octadecenoic acid ($C_{18:1}$); 3-hydroxydecanoic acid ($C_{10:0\ 3OH}$) is always present. **The major quinone is ubiquinone Q-8.**

The mol% G + C of the DNA is: 60–69.

Type species: **Comamonas terrigena** (ex Hugh 1962) De Vos, Kersters, Falsen, Pot, Gillis, Segers and De Ley 1985b, 450, emend. Willems, Pot, Falsen, Vandamme, Gillis, Kersters and De Ley 1991c, 439.

FURTHER DESCRIPTIVE INFORMATION

Cell morphology Cells are rods that are straight or slightly curved in one plane or spirilla. Polar pili have been reported in *Comamonas testosteroni* (Fuerst and Hayward, 1969b).

Cell wall composition Cell walls have a typical Gram-negative structure consisting of an outer membrane and a cytoplasmic membrane, separated by a dense peptidoglycan layer. Analysis of the lipid A component of the lipopolysaccharides in the outer membrane of *C. testosteroni* has revealed the lipid A backbone to consist of 6-*O*-(2-deoxy-2-amino-β-D-glucopyranosyl)-2-deoxy-2-amino-α-D-glucose, which is phosphorylated in positions 1 and 4′. The hydroxyl groups at positions 4 and 6′ are unsubstituted, and position 6′ of the non-reducing terminal residue is the attachment side for the polysaccharide component. The 2, 2′, 3, and 3′ positions of the sugar backbone are *N*-acylated or *O*-acylated by 3-hydroxydecanoic acid, and the hydroxyl groups of the amide-linked residues at positions 2 and 2′ of the backbone are *O*-acylated by tetradecanoic and dodecanoic acids, respectively (Iida et al., 1996).

Colonial characteristics On nutrient agar, colonies are round and convex with a smooth to wavy margin and a smooth to granular surface. Most strains are unpigmented, but some strains may produce a brown diffusible pigment on nutrient agar. Colony diameters can reach 0.4–3 mm after 3 d at 28°C. Occasionally, two colony types, one mucoid and the other non-mucoid, can be isolated from one strain. Comparison of such types by whole-cell protein electrophoresis shows identical protein profiles, and they are therefore considered morphological variants of the same strain (Willems et al., 1991c). For a *C. testosteroni* strain isolated from activated sludge on the basis of its ability to coaggregate with yeast cells, two colony types have been reported to occur together on solid agar media, resulting in composite colonies with sectors of different morphologies (Bossier and Verstraete,

1996). In liquid medium, in the absence of agitation, and in contact with glass, cultures shift towards and are dominated by mucoid-colony-forming cells. In such media, the addition of stress factors such as hydrogen peroxide, sodium dodecyl sulfate, or starvation results in the rapid formation of non-mucoid cells. Non-mucoid cell types rapidly coaggregate and settle in flocs with yeast cells, whereas mucoid cells do not coaggregate with yeast cells (Bossier and Verstraete, 1996).

Nutrition and growth conditions *Comamonas* strains grow well on most organic acids and amino acids but use very few sugars (De Vos et al., 1985b). They are capable of degrading a wide variety of complex aromatic compounds, steroids, and many man-made complex organic molecules. According to Tamaoka et al. (1987), *C. terrigena* requires methionine and nicotinamide as growth factors in a mineral medium with L-glutamate as the carbon source. Optimal growth temperature is 30°C.

Metabolism *Comamonas* strains have a respiratory metabolism using oxygen as the terminal electron acceptor. Although most *Comamonas* strains are capable of nitrate reduction, most cannot reduce nitrites. Only 3 of 42 *C. terrigena* strains have tested positive for nitrite reduction (Willems et al., 1991c). In a study of the denitrifying biofilms at a water treatment plant, *Comamonas* strains were identified, but they were thought to have washed in from previous stages and to not be true members of the denitrifying biofilms (Lemmer et al., 1997). An isolate named *Comamonas* strain SGLY2, capable of aerobic denitrification and nitrogen production without nitrite build-up, has been studied for application in mixed-culture aerated reactors (Patureau et al., 1997).

C. testosteroni strains capable of degrading polycyclic aromatic hydrocarbons, such as phenanthrene, naphthalene, and anthracene, have been described (Goyal and Zylstra, 1996). Their genes for naphthalene and phenanthrene degradation have been cloned and characterized and found to differ from the classical naphthalene degradation genes (*nah*) of *Pseudomonas putida*. Among different *C. testosteroni* strains, at least two different sets of genes for phenanthrene degradation may be present. In addition, in one of the strains, the arrangement of these genes differs from that in *Pseudomonas* species (Goyal and Zylstra, 1996).

The gene *phtD* for 4,5-dihydroxyphthalate decarboxylase, an enzyme involved in phthalate metabolism, has been identified and sequenced. The deduced amino acid sequence shows more than 77% similarity to the *pht5* gene from *Pseudomonas putida*. The sequence upstream of *phtD* and its deduced amino acid sequence show high similarity to *pht1* and its deduced amino acid sequence, which is thought to be the positive regulator for the other *pht* genes of *Pseudomonas putida*. These data suggest a common origin for the genes involved in phthalate metabolism (Lee et al., 1994c).

C. testosteroni uses the meta cleavage pathway for the degradation of 4-chlorophenol and related compounds, as has been demonstrated by the isolation and identification of several of the key enzymes and intermediary metabolites of this pathway (Hol-

lender et al., 1997). The *bphD* gene from strain B-356, encoding for 2-hydroxy-6-oxo(phenyl/chlorophenyl) hexa-2,4-dienoic acid hydrolase has been sequenced, and the gene product has a mechanism of action similar to that of classical lipases and serine hydrolases (Ahmad et al., 1995).

The first step in the degradation of biphenyl and chlorobiphenyl compounds by *C. testosteroni* is their conversion to 2,3-dihydro-2,3-dihydroxybiphenyl by biphenyl/chlorobiphenyl dioxygenase. This enzyme system consists of three components, the genes of which have been sequenced: a terminal oxygenase, which is an Fe–S protein consisting of 2 subunits (encoded by *bphA* and *bphE*); a ferredoxin, encoded by *bphF*; and a ferredoxin reductase encoded by the *bphG* gene, which is not located near the *bphAEF* genes in strain B-356 (Sylvestre et al., 1996b). Most of these enzymes or recombinant forms of them have been characterized in some detail (Hurtubise et al., 1995, 1996). Further degradation steps involve 2,3-dihydro-2,3-dihydroxybiphenyl dehydrogenase (*bphB*), a recombinant form of which has been characterized (Sylvestre et al., 1996a), and 2,3-dihydroxybiphenyl-1,2-dioxygenase (*bphC*), which catalyzes *meta*-1,2 fission of the aromatic ring (Bergeron et al., 1994).

In *Comamonas* sp. strain JS765, nitrobenzene is broken down by nitrobenzene 1,2-dioxygenase to nitrohydrodiol, which spontaneously decomposes into catechol and nitrite. Catechol is then broken down via the meta cleavage pathway. The catechol 2,3-dioxygenase gene *cdoE*, a ferredoxin gene *cdoT*, and a regulatory gene *cdoR* from strain JS765 have been sequenced and their amino acid sequences deduced (Parales et al., 1997).

Experimental evidence indicates that the mechanism of uptake of 4-toluene sulfonate by *C. testosteroni* strain T-2 involves an inducible secondary proton symport system (Locher et al., 1993). Strain T-2 degrades 4-toluene sulfonate and 4-toluene carboxylate by oxygenation of the side chains to 4-sulfobenzoate and terephthalate, respectively, before further oxidation to protocatechuate. The first three enzymes involved in this pathway are a 4-toluenesulfonate methyl-monooxygenase system, which consists of a reductase B and an oxygenase M (*tsaMB* genes), a 4-sulfobenzyl alcohol dehydrogenase (*tsaC*), and a 4-sulfobenzaldehyde (*tsaD*). Their genes are coexpressed under the regulation of *tsaR*. The components of this system have been purified and partially characterized. The *tsaMB* oxygenase system is a class IA mononuclear iron oxidase, with *tsaM* having a Rieske [2Fe–2S] center. *TsaC* is a short-chain zinc-dependent dehydrogenase (Junker et al., 1997). The conversion of 4-sulfobenzoate to protocatechuate requires a 4-sulfobenzoate-3,4-dioxygenase system encoded by the *psbAC* genes and consisting of a reductase C and an oxygenase A, which again contains a Rieske [2Fe–2S] center (Junker et al., 1996). Both sets of genes (*tsaMBCD*, *tsaR*, and *psbAC*) are located on conjugative plasmids in some *C. testosteroni* strains (Junker and Cook, 1997). The degradation of 4-toluene sulfonate by two different strains is comparable in liquid cultures but varies in biofilms (Khlebnikov et al., 1997). Terephthalate is broken down by a terephthalate dioxygenase system to (1*R*,2 *S*)-dihydroxy-3,5- cyclohexadiene-1,4-dicarboxylic acid, which is further degraded by (1*R*,2 *S*)-dihydroxy-1,4-dicarboxy-3,5-cyclohexadiene dehydrogenase to protocatechuate (Oppenberg et al., 1995). The dioxygenase system is a Rieske [2Fe–2S] protein consisting of two subunits, α and β, which are thought to form an $\alpha_2 \beta_2$ structure (Schläfli et al., 1994). Protocatechuate is further degraded via the meta cleavage pathway. Enzymes involved in these processes are thought to be located on the chromosome (Junker and Cook, 1997).

Metabolism of steroids　　Möebus et al. (1997) have observed that testosterone, used as a sole carbon source by *Comamonas testosteroni*, induces expression of steroid- and aromatic hydrocarbon-catabolizing enzymes and represses at least one amino acid degrading enzyme. It has been suggested that steroids may play a regulative role in catabolic enzyme synthesis in adaptive growth. Several enzymes involved in the steroid metabolism of *C. testosteroni* have been studied. A 3-α-hydroxysteroid dehydrogenase has been isolated and characterized (Oppermann and Maser, 1996), and its gene has been sequenced, cloned and expressed (Abalain et al., 1995). Similarity to ribosomal proteins L10 and L7/12 has led to the suggestion that this enzyme may be formed by fusion of two ribosomal proteins (Baker, 1996). The gene for 3-ketosteroid-δ^4-5-α-dehydrogenase has been sequenced and is located downstream of a gene for 3-ketosteroid-δ^1-dehydrogenase. The gene product is a flavoprotein. Although both of these dehydrogenases are functionally similar, their genes are probably not derived from a common ancestor (Florin et al., 1996). The 3β/17β hydroxysteroid dehydrogenase of *Comamonas testosteroni* has been studied by mutagenic replacement within the active site to identify important residues (Oppermann et al., 1997). The structure and function of δ^5-3-ketosteroid isomerase and 3-oxo-δ^5-steroid isomerase, which catalyze the conversion of δ^5- to δ^4-3-ketosteroids by an intramolecular proton transfer, have been studied extensively (Brothers et al., 1995; Zhao et al., 1996, 1997). A bile acid 3-α-sulfate sulfohydrolase from *Comamonas testosteroni* has been purified and characterized (Tazuke et al., 1994).

Metabolism of other organic compounds　　*Comamonas* strains capable of degrading terpenes, such as abietane and pimarane-type resin acids, have been isolated by enrichment from wastewater from an aerated stabilization basin of a bleached kraft pulp mill (Morgan and Wyndham, 1996).

Extracellular poly-β-hydroxybutyrate depolymerase has been purified from a *Comamonas* sp. strain. In addition to poly-β-hydroxybutyrate, it can also hydrolyze poly(β-hydroxybutyrate-co-β-hydroxyvalerate) and poly-β-hydroxyvalerate. It differs from other poly-β-hydroxybutyrate depolymerases in that it is insensitive to phenylmethylsulfonyl fluoride and that it hydrolyzes poly-β-hydroxybutyrate to β-hydroxybutyrate monomers (Jendrossek et al., 1993). Part of the encoding gene has been cloned and sequenced, and the deduced protein structure has been comparatively analyzed (Jendrossek et al., 1995). Shinomiya et al. (1997) have since cloned, sequenced, and analyzed a gene for the same enzyme from *C. testosteroni* YM1004 and obtained a very similar sequence.

An aliphatic nitrilase, active on adiponitrile and cyanovaleric acid, has been isolated and purified from *Comamonas testosteroni*, and its gene has been sequenced and overexpressed. The Cys163 residue is thought to play an essential role in the active site (Levy-Schil et al., 1995).

A quinohemoprotein ethanol dehydrogenase, which catalyzes the NAD-independent oxidation of a broad range of alcohols to the corresponding aldehydes and on to the carboxylic acids, has been isolated and purified from *Comamonas testosteroni* cells grown on ethanol. The active holoenzyme has been found to contain pyrroquinoline–quinone, calcium ions, and heme c (De Jong et al., 1995). The encoding gene (*qhedh*) has been cloned, sequenced, and expressed in *E. coli* (Stoorvogel et al., 1996). The enzyme has been investigated for use in the production of (*S*)-solketal (2,2-dimethyl-1,3-dioxolane-4-methanol) by enantio-

selective oxidation of racemic solketal (Geerlof et al., 1994a) and has been applied in electrodes through immobilization in a redox polymer network (Stigter et al., 1997). Quinoline 2-oxidoreductase and 2-oxo-1,2-dihydroquinoline 5,6-dioxygenase, the enzymes involved in the first two steps of the degradation of quinoline and 3-methyl quinoline, have been isolated from *Comamonas testosteroni* strain 63 and their structure and cofactors studied. Quinoline 2-oxidoreductase is a molybdo-iron/sulfur flavoprotein, and 2-oxo-1,2-dihydroquinoline 5,6-dioxygenase is a single component protein (Schach et al., 1995).

The iron-containing nitrile hydratase from *C. testosteroni* strain Nil, which catalyzes the hydration of 5-cyanovaleric acid to adipamic acid, is inactivated by stoichiometric amounts of NO, and is reactivated by photoirradiation. In these reactions, it is similar to a *Rhodococcus* R312 nitrile hydratase, which has a quite different amino acid sequence (Bonnet et al., 1997).

5-Aminolevulinic acid, a precursor to tetrapyrroles, is formed from aminoacylated tRNA–Glu in a two-step pathway by *C. testosteroni*. This pathway involves glutamyl–tRNA reductase, encoded by *hemA*, and glutamine-1-semialdehyde-2,1-aminomutase, encoded by *hemL* (Hungerer et al., 1995). In a study on the synthesis of new agricultural chemicals, *Comamonas testosteroni* strain CMI 2848 possessed the highest activity for hydroxylating 3-cyanopyridine to 3-cyano-6-hydroxypyridine among 4600 isolates screened (Yasuda et al., 1995).

C. testosteroni is capable of selectively degrading L-lysine from racemized lysine crystals, a characteristic which has been applied in the large-scale production of D-lysine (Takahashi et al., 1997).

The various pathways for the biosynthesis of aromatic amino acids and their regulation have been investigated extensively among pseudomonad bacteria. *C. testosteroni* possesses prephenate dehydrogenase and arogenate dehydrogenase reactive with either NAD or NADP. Arogenate dehydratase activity is absent; prephenate dehydratase is present, and its activity is increased 6–12-fold by L-tyrosine (Byng et al., 1983).

Chemotaxonomic data *Comamonas* strains contain ubiquinone Q-8 as the major quinone (>90%), with smaller amounts of Q-7 and Q-9 (Tamaoka et al., 1987). Major fatty acids (comprising 10–40% of the total amount) are palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), and *cis*-vaccenic acid ($C_{18:1\ \omega7c}$). Small amounts (2–7%) of lauric acid ($C_{12:0}$) are present. Myristic acid ($C_{14:0}$) is present as 3–4% of the total in *C. terrigena* and less than 1% of the total in *C. testosteroni*. 2-Hydroxyhexadecanoic acid ($C_{16:0\ 2OH}$) is present as less then 1% of the total in *C. terrigena* and 2–7% in *C. testosteroni* (Tamaoka et al., 1987; Willems et al., 1989). For some fatty acids, the amounts reported by Tamaoka et al. (1987) and Willems et al. (1989) are quite different: 3-hydroxydecanoic acid ($C_{10:0\ 3OH}$), 3–5% and 7–17%, respectively; *n*-heptadecanoic acid ($C_{17:0}$), <2% and traces to 7%; cyclopropane-substituted methylene-hexadecanoic fatty acid ($C_{17:0\ cyclo}$), <2% and 1–15%. The polyamine content of *C. testosteroni* strain DSM 1622 has been determined. The major polyamines (>50%) are 2-hydroxyputrescine and putrescine; spermidine, spermine, and 1,3-diaminopropane are present in much smaller quantities (<5%). Cadaverine has not been detected (Busse et al., 1992).

Plasmids Plasmids have been isolated from and successfully transferred to *Comamonas* strains and may contribute to the catabolic versatility and flexibility of these organisms. *C. testosteroni* strains T-2, PSB-4, and the type strain have been reported to contain two plasmids (pTSA and pT2T), one plasmid (pPSB), and no plasmids, respectively (Junker and Cook, 1997). Plasmid

pTSA (85 kb) is a conjugative plasmid belonging to the IncP1 group. It carries the genes *tsaMBCD*, *tsaR*, and *psbAC*, involved in the degradation of 4-toluene sulfonate to protocatechuate, as well as two copies of insertion element IS 1071. Plasmid pPSB (85 kb) is also a conjugative plasmid and carries the *psbAC* genes, as well as two copies of IS 1071. Results of conjugation experiments with the type strain suggest that the *psb* genes are located in a composite transposon (Junker and Cook, 1997).

Comamonas strains have been used in various experiments as recipients of plasmids carrying catabolic genes. Plasmid RP4::Tn4371, carrying genes for biphenyl and 4-chlorobiphenyl degradation, has been transferred from *Enterobacter agglomerans* to indigenous soil bacteria, including *Comamonas* sp., where these catabolic genes are expressed (De Rore et al., 1994). Plasmid PR4::Mu3A, carrying chromosomal DNA fragments encoding the ability to use biphenyl as the sole carbon source, has been transferred to *C. testosteroni* and the catabolic genes effectively expressed (Springael et al., 1996). The plasmids pACK5 and pACT72, which each include a replicon of the cryptic plasmid pAC1 from *Acetobacter pasteurianus*, have been transferred to and successfully expressed in *Comamonas terrigena*, as well as in several other Gram-negative and Gram-positive taxa (Grones and Turna, 1995).

Antibiotic sensitivity *C. testosteroni* possesses steroid-inducible hydroxysteroid dehydrogenases/carbonyl reductases. This contributes to increased resistance of cells grown on a steroid substrate to the fungal steroid fusidic acid and to faster uptake and alternative metabolism of the anti-insect agent NKI 42255 (2-(1-imidazolyl)-1-(4-methoxyphenyl)-2-methyl-1-propane). These steroid-inducible pathways provide protection against natural and synthetic toxic compounds present in the soil and in the intestinal tracts of mammals (Oppermann et al., 1996).

Ecology *Comamonas* strains have been isolated from sites heavily contaminated with various complex organic compounds and heavy metals. Resistance of *Comamonas* strains to a number of heavy metals has been reported. A cadmium-resistant strain of *C. testosteroni* has been isolated from soil contaminated with heavy metals (Kanazawa and Mori, 1996), and nickel-resistant *Comamonas* strains have been isolated from naturally nickel-percolated soils from New Caledonia (Stoppel and Schlegel, 1995). Among other chromate-reducing bacteria isolated from the cooling water of an electricity generating plant, a strain tentatively identified as *Comamonas testosteroni* has been found to be one of the strongest chromate-reducers and has therefore been implicated in blockage of pipes due to precipitation of chromium (III) oxide (Cooke et al., 1995).

Because of their ability to degrade a wide variety of complex organic compounds, *Comamonas* strains are of potential interest in bioremediation. *C. testosteroni* has been used to degrade 4-toluenesulfonic acid in a continuously operated fixed-bed biofilm reactor (Khlebnikov and Peringer, 1996). *Comamonas* strains are among the rare isolates of the *Betaproteobacteria* that have been identified in activated sludge (Kämpfer et al., 1996). Cells of *C. terrigena* strain N3H are capable of degrading the anion active surfactant dihexyl-sulfosuccinate and have been applied for this purpose immobilized in alginate gel (Huska et al., 1996). Cells starved of a carbon source for 16 h have the highest biotransformation rate of dihexyl-sulfosuccinate (Toth et al., 1996).

Among isolates from a PCB-polluted site, *C. testosteroni* strains comprise the majority and are capable of degrading biphenyl and various polychlorinated biphenyls (Joshi and Walia, 1995).

Comamonas strains that degrade poly-β-hydroxybutyrate (PHB) have frequently been isolated from various environments (Mergaert and Swings, 1996), and the extracellular PHB depolymerase of one strain has been purified and characterized (Jendrossek et al., 1993).

Because of the broad substrate specificity of its 3-α-hydroxysteroid dehydrogenases of *C. testosteroni* and the occurrence of this and related bacterial species in the intestinal tract of vertebrates, it has been suggested that these enzymes may contribute to bioactivation or inactivation of hormones, bile acids, and xenobiotics (Oppermann and Maser, 1996).

ENRICHMENT AND ISOLATION PROCEDURES

Comamonas strains are common inhabitants of soil, mud, and water, in both natural and polluted environments. They have also been isolated from various clinical samples, the hospital environment, and horse and rabbit blood (Willems et al., 1991c). *Comamonas* strains can generally be isolated from water by plating on nutrient agar, but specific selective isolation procedures yielding only *Comamonas* isolates have not been described. The original *C. terrigena* type strain was isolated on a medium of hay infusion filtrate (Mudd and Warren, 1923). Often, selective enrichment can be obtained by targeting the capacity of *Comamonas* strains to degrade particular aromatic compounds, hydrocarbons, and higher dicarboxylic acids by using these compounds as the sole carbon source in a mineral medium. Tamaoka et al. (1987) reported that *C. terrigena* requires methionine and nicotinamide as growth factors and, consequently, these should be added to isolation media.

The following compounds have been used to isolate or enrich for *Comamonas* strains: phenol and *m*-cresol (Gray and Thornton, 1928), abietic and dehydroabietic acid (Morgan and Wyndham, 1996), and poly-β-hydroxybutyrate (Jendrossek et al., 1993). The isolation of *C. testosteroni* strains has been reported with the following compounds: testosterone (Talalay et al., 1952), imidazolylpropionate and imidazolyl-lactate (Coote and Hassal, 1973), *p*-cresol (Dagley and Patel, 1957), fumarate, bromosuccinate, anthranilate, kynurenate, and poly-3-hydroxybutyrate (Stanier et al., 1966), naphthalene (García-Valdés et al., 1988), phenanthrene (Goyal and Zylstra, 1996), chlorophenol and methylphenol (Hollender et al., 1997), and polychlorinated biphenyls (Joshi and Walia, 1995). *C. testosteroni* strains have also been isolated by selecting for cadmium resistance (Kanazawa and Mori, 1996).

Comamonas strains have been isolated increasingly from the clinical environment (Gilardi, 1971, 1985; Ben-Tovim et al., 1974; De Vos et al., 1985b; Willems et al., 1991c) and are regarded as rare opportunistic pathogens (Gilardi, 1985). Sources include blood, pus, urine, pharyngeal mucosae, kidneys, feces, burst appendix, intravenous tubing, and urinary catheters. *Comamonas* strains are isolated from such samples using methods for the isolation of Gram-negative glucose-nonfermenters. This includes the use of a blood agar medium, such as tryptic soy agar plus defibrinated blood, and a selective enteric medium, such as MacConkey agar. The commonly used incubation regime for primary isolation media of 24 h at 35°C should be extended with 24 h at 30°C to permit growth of glucose-nonfermenters that grow slowly at 35°C and may be masked by other bacteria (Rubin et al., 1985).

MAINTENANCE PROCEDURES

Comamonas strains can be maintained on nutrient agar at 4°C for up to 2 months. For long-term preservation, strains can be lyophilized using standard procedures. This can be done routinely by freeze-drying the cells in horse serum (70%) supplemented with glucose (7%) and nutrient broth (0.6%).

DIFFERENTIATION OF THE GENUS *COMAMONAS* FROM OTHER GENERA

Table BXII.β.42 lists features differentiating the genus *Comamonas* from other genera in the family.

Restriction analysis of a 2.4 kb amplified ribosomal DNA fragment containing the 16S rRNA gene, the spacer region between 16S and 23S genes, and part of the 23S rRNA gene with the enzyme *Hin*f1 allows differentiation of *Comamonas* species from members of *Acidovorax*, *Delftia* (formerly *Comamonas acidovorans*), *Hydrogenophaga*, and *Variovorax* (Vaneechoutte et al., 1992).

An oligonucleotide probe targeting 16S rRNA has been used to identify members of the genus *Comamonas*, among other members of the *Comamonadaceae* (Amann et al., 1996). Its sequence is 5′-ACCTACTTCTGGCGAGA-3′, which is homologous to positions 1424–1440 on the rRNA.

TAXONOMIC COMMENTS

The nomenclatural history of the genus *Comamonas* is complex and was initially quite obscure because of incomplete descriptions and lack of original cultures. *Comamonas* was therefore not included in the Approved Lists of Bacterial Names (Skerman et al., 1980), nor was it listed in the previous edition of this *Manual*, although in the 8th edition of *Bergey's Manual of Determinative Bacteriology*, *Comamonas terrigena*, "*Vibrio cyclosites*", and "*Vibrio neocistes*" were included as *species incertae sedis* in addendum IV of the genus *Pseudomonas*. The genus became valid only in 1985, when the name was revived (De Vos et al., 1985b).

The name *Comamonas* was originally proposed by Davis and Park (1962) to replace the invalid name *Lophomonas*. This latter genus, with one species (*Lophomonas alcaligenes*), had been proposed for a group of Gram-negative rod-shaped bacteria attacking few carbohydrates and possessing 2–4 lophotrichous flagella (Galarneault and Leifson, 1956). *L. alcaligenes* was considered to be a subjective synonym of the peritrichously flagellated *Vibrio alcaligenes*, which had itself been created (Lehmann and Neumann, 1927) to replace *Bacillus faecalis alcaligenes*, a species which contained Gram-negative, rod-shaped, human fecal isolates that did not attack carbohydrates (Petruschky, 1896). When the genus *Comamonas* was created to replace *Lophomonas*, the former type species (*Lophomonas alcaligenes*) was not maintained because of differences between the original descriptions of *Vibrio alcaligenes* and the new genus *Comamonas*. Instead, *Comamonas percolans*, formerly *Vibrio percolans* (Mudd and Warren, 1923), was proposed as the type species. *Vibrio alcaligenes*, *Vibrio neocistes*, and *Vibrio cyclosites* were also assigned to the new genus *Comamonas*, but were not allocated to a particular species (Davis and Park, 1962). *Comamonas percolans* was eventually shown to be a later subjective synonym of *Vibrio terrigenus*, an organism isolated from soil and motile by means of bipolar tufts of flagella (Günther, 1894), and the organism was therefore renamed *Comamonas terrigena* (Hugh, 1962). Later still, Hugh (1965) reported that the type strains of *Comamonas terrigena* and *Pseudomonas testosteroni* were very similar and suggested both species be united.

The genus *Comamonas* was revived in 1985 (De Vos et al., 1985b) after a polyphasic study that included some of the old preserved isolates. Initially, only four strains, each motile by a polar tuft of flagella, were included in the revived species *Comamonas terrigena*: NCIB 8193^T (formerly *Vibrio percolans*), NCIB

2581 (formerly *Vibrio cyclosites*), NCIB 2582 (formerly *Vibrio neocistes*) and CCUG 12940. Later, *Pseudomonas acidovorans* and *Pseudomonas testosteroni* were transferred to an emended genus *Comamonas* as *Comamonas acidovorans* and *Comamonas testosteroni* (Tamaoka et al., 1987). A polyphasic study that included many unnamed clinical isolates, as well as strains representing related genera, resulted in the inclusion of many more strains in *C. terrigena* and the recognition of three subgroups in this species. These subgroups can be distinguished by DNA–rRNA and DNA–DNA hybridizations, whole cell protein electrophoresis, and immunotyping, but not by morphological, auxanographic, or biochemical characterization; consequently, they have been retained in a single species (Willems et al., 1991c). The first subgroup contains the four strains assigned to *C. terrigena* by De Vos et al. (1985b) and one additional clinical strain. The second subgroup comprises the type strain of *Aquaspirillum aquaticum* and 13 clinical isolates previously designated EF group 10. The third subgroup comprises 13 clinical strains of EF group 10 as well as three misnamed *Pseudomonas* strains from blood. Several new *Comamonas terrigena* strains cannot be assigned to any of the three groups, indicating that this species may contain even more diversity than was previously thought (Willems et al., 1991c). The name *Aquaspirillum aquaticum* was abandoned since it was a later synonym of *Comamonas terrigena*. Since this organism has bipolar tufts of flagella, its inclusion in *Comamonas* brought the genus description, at least for this aspect of morphology, back in line with the original description of *Vibrio terrigenus* (Günther, 1894).

From DNA–rRNA hybridizations it was apparent that the three *C. terrigena* subgroups and *C. testosteroni* were probably more closely related to each other than to *C. acidovorans* (Willems et

al., 1991c). This has recently been confirmed by 16S rRNA gene sequence analysis and, consequently, it has been proposed that *C. acidovorans* be transferred to a new genus *Delftia* as *Delftia acidovorans* (Wen et al., 1999). By 16S rDNA analysis, *C. terrigena* and *C. testosteroni* cluster together, but their grouping is not supported by a high bootstrap value. This indicates that the grouping of these branches is not very stable, and their position in the dendrogram may change as sequence data for additional strains are included. It is therefore at present not clear whether both species form a phylogenetically coherent genus, comparable to, for example, *Hydrogenophaga* or *Acidovorax*.

Comamonas spp. are regularly reported in ecological or applied studies involving isolates from soil and water. Although some of these isolates may escape species identification because of inadequacy of the identification methods used, other isolates may represent additional species within the genus. Wen et al. (1999) have described a *Comamonas* strain with an taxonomic position that is intermediate between *C. terrigena* genomic subgroup 1 (*sensu* Willems et al., 1991c) and *C. testosteroni*, but no representatives of the other two genomic subgroups were included in their analysis. It is possible, therefore, that this strain may belong to one of these two *C. terrigena* genomic subgroups.

"*Comamonas compransoris*", which has been described as a facultatively lithotrophic carbon monoxide or hydrogen oxidizer (Nozhevnikova and Zavarzin, 1974), has been shown to belong to the *Alphaproteobacteria* and has been assigned to the new genus *Zavarzinia* as *Zavarzinia compransoris* (Meyer et al., 1993).

DIFFERENTIATION OF THE SPECIES OF THE GENUS *COMAMONAS*

Table BXII.β.44 lists characteristics differentiating *Comamonas terrigena* from *Comamonas testosteroni*.

It is possible to distinguish the three *C. terrigena* groups, as well as *C. testosteroni*, by restriction analysis with the enzyme *Hin*f1 of a 2.4 kb amplified ribosomal DNA fragment containing the 16S rRNA gene, the spacer region between 16S and 23S genes, and part of the 23S rRNA gene (Vaneechoutte et al., 1992).

TABLE BXII.β.44. Characteristics differentiating *Comamonas* species[a,b]

Characteristic	*C. terrigena*	*C. testosteroni*
Occurrence of 2-hydroxy fatty acids	−	+
Growth on:		
L-Histidine	−	+
2-Aminobenzoate (anthranilate)	−	+
Citrate	−	+
Glycolate	−	+
Benzoate	−	+[c]
Testosterone	−	+

[a]For symbols see standard definitions.

[b]Data taken from Palleroni (1984), De Vos et al. (1985b), Tamaoka et al. (1987), and Willems et al. (1991c).

[c]Negative according to Tamaoka et al. (1987); 11–89% of strains are positive (d score) according to Palleroni (1984).

List of species of the genus Comamonas

1. **Comamonas terrigena** (ex Hugh 1962) De Vos, Kersters, Falsen, Pot, Gillis, Segers and De Ley 1985b, 450[VP] emend. Willems, Pot, Falsen, Vandamme, Gillis, Kersters and De Ley 1991c, 439.
   ter.ri.ge′ na. L. n. *terra* soil; L. v. *gignere* to bear; L. n. *terrigena* borne by the earth (soil), child of the earth.

   The characteristics are as described for the genus and as indicated in Tables BXII.β.44 and BXII.β.45.

   The type strain was isolated from a hay infusion filtrate.
   The mol% G + C of the DNA is: 59.7–63.3 (species); 64 (type strain) (T_m).

   Type strain: ATCC 8461, IAM 12409, LMG 1253, NCIB 8193.
   GenBank accession number (16S rRNA): AB021418.

2. **Comamonas denitrificans** Gumaelius, Magnusson, Petterson and Dalhammar 2001, 1005[VP]
   de.ni.tri′ fi.cans. L. prep. *de* away from; L. n. *nitrum* soda; N.L. n. *nitrum* nitrate; M.L. v. *denitrifico* to denitrify; N.L. part.adj. *denitrificans* denitrifying.

   The characteristics are the same as those of the genus. This species is not included in Tables BXII.β.44 and BXII.β.45 because it was published after the completion of

TABLE BXII.β.45. Additional characteristics of *Comamonas* species[a,b]

Characteristic[c]	*C. terrigena*	*C. testosteroni*
Growth at 4°C	nd	−
Growth at 42°C	d	−
Growth with 3% NaCl	d	d
Growth with 4.5% NaCl	d	−
Susceptibility to penicillin (10 μg/disk)	d	−
Growth on cetrimide	−	d
Hydrolysis of Tween 80	−	d[d]
Christensen urease	−	d
Phosphoamidase	+	d
Nitrate reduction	+	d
Denitrification	nd	−
Chemolithoautotrophic growth with H₂ as electron donor	nd	−
Levan from sucrose	nd	−
Poly-β-hydroxybutyrate accumulation	nd	+
Extracellular poly-β-hydroxybutyrate hydrolysis	nd	d
Growth on:		
Ethanol	nd	−
Glycerol	−	d[e,f]
Gluconate	d	+
Ethylene glycol, propylene glycol, 2,3-butylene glycol, *n*-hexadecane	nd	−
ᴅʟ-Kynurenine	−	d
ʟ-Threonine, ʟ-tryptophan	−	d[e,f]
Glycine	−	d[d,g]
ᴅ-α-Alanine	d	d[d]
ʟ-Alanine, ʟ-valine, ᴅʟ-norvaline, ᴅʟ-norleucine	d	d
ᴅʟ-4-aminobutyrate	−	−[h]
ʟ-Isoleucine, ʟ-norleucine, ʟ-tyrosine	d	+
ʟ-Phenylalanine	d	+[i]
ʟ-Asparagine, glucono-δ-lactone	nd	+
ᴅʟ-Methionine, putrescine	nd	−
Acetate, succinate	+	+[i]
Isobutyrate	+	+[h]
Heptanoate, caprate	d	d[f]
Pyruvate, aconitate	d	d[d]
Mesaconate	d	d
Caprylate	d	d[e,f]
ʟ-Tartrate, ʟ-mandelate	−	d[f]
m-Tartrate, isophthalate, terephthalate	−	d
Phthalate	−	d[e]
Pelargonate	−	d[e,f]
ᴅ-Tartrate, trigonelline	−	−[h]
ᴅʟ-Glycerate, citraconate, itaconate, *m*-hydroxybenzoate, *p*-hydroxybenzoate	d	+
α-Ketoglutarate	d	+[e]
Saccharate, mucate, hydroxymethylglutarate, butanol, ʟ-kynurenine, kynurenate	nd	+
ᴅʟ-Malate, hippurate, *n*-propanol, geraniol, poly-β-hydroxybutyrate, benzoylformate, 2-aminovalerate, nicotinate	nd	d
Phenol	nd	d[f]
ᴅʟ-Tartrate, anthranilate, isobutanol, phenylethanediol, naphthalene, pantothenate, dodecane, hexadecane, quinate	nd	−
Hydrolysis of:		
2-Naphthylphosphate (pH 5.4)	d	d
2-Naphthylcaprylate	d	+
Naphthol-AS-BI-phosphodiamide	+	d

[a]For symbols see standard definitions; nd, not determined.

[b]Data taken from Palleroni (1984), De Vos et al. (1985b), Tamaoka et al. (1987), and Willems et al. (1991c).

[c]The following characteristics are present in both species: growth at 30 and 37°C, growth on Drigalski-Conradi agar; growth in the presence of 0.5 or 1.5% NaCl; growth on ʟ-proline, ʟ-leucine, ʟ-aspartate, ʟ-glutamate, propionate, butyrate, *n*-valerate, levulinate, isovalerate, *n*-caproate, ᴅ-malate, fumarate, glutarate, adipate, pimelate, suberate, azelate, sebacate, ᴅʟ-lactate, ᴅʟ-β-hydroxybutyrate, and ʟ-malate; and hydrolysis of 2-naphthylbutyrate, ʟ-leucyl-2-naphthylamide. The following characteristics are absent in both species: growth in the presence of 6.5% NaCl; acid production in 10% lactose, in triple sugar iron medium, and in OF medium with ᴅ-glucose, ᴅ-fructose, ᴅ-xylose, maltose, or adonitol; α-hemolysis; hydrolysis of esculin, gelatin, starch, acetamide, and DNA; lysine and ornithine decarboxylase; arginine dihydrolase; indole production; nitrite reduction; and β-galactosidase. Both species fail to grow on ᴅ-arabinose, ʟ-arabinose, ᴅ-lyxose, ᴅ-ribose, ᴅ-xylose, ʟ-xylose, ᴅ-fructose, ʟ-fucose, ᴅ-fucose, ᴅ-tagatose, adonitol, ᴅ-arabitol, ʟ-arabitol, *m*-xylitol, *m*-erythritol, *m*-inositol, ᴅ-mannitol, ᴅ-galactose, ᴅ-glucose, ᴅ-mannose, ʟ-rhamnose, ʟ-sorbose, dulcitol, sorbitol, ᴅ-cellobiose, β-gentiobiose, lactose, maltose, ᴅ-melibiose, sucrose, trehalose, ᴅ-turanose, ᴅ-melezitose, ᴅ-raffinose, methyl-β-ᴅ-xyloside, methyl-α-ᴅ-glucoside, methyl-α-ᴅ-mannoside, 2-ketogluconate, 5-ketogluconate, *N*-acetylglucosamine, amygdalin, arbutin, salicin, esculin, glycogen, inulin, starch, ʟ-cysteine, ʟ-ornithine, ʟ-lysine, ʟ-citrulline, β-alanine, ʟ-serine, ʟ-methionine, ᴅ-tryptophan, ʟ-arginine, ᴅʟ-2-aminobutyrate, ᴅʟ-3-aminobutyrate, ᴅʟ-5-aminovalerate, 3-aminobenzoate, 4-aminobenzoate, ethanolamine, ethylamine, butylamine, amylamine, benzylamine, acetamide, diaminobutane, urea, betaine, creatine, spermine, sarcosine, histamine, tryptamine, glucosamine, oxalate, maleate, malonate, phenylacetate, *o*-hydroxybenzoate, or ᴅ-mandelate. Neither species hydrolyzes 2-naphthylmyristate, 2-naphthylphosphate (pH 8.5), ʟ-cystyl-2-naphthyl-amide, ʟ-valyl-2-naphthylamide, *N*-benzoyl-ᴅʟ-arginine-2-naphthylamide, *N*-glutaryl-phenylalanine-2-naphthylamide, 6-bromo-2-naphthyl-α-ᴅ-galactopyranoside, 2-naphthyl-β-ᴅ-galactopyranoside, naphthol-AS-BI-β-ᴅ-glucuronate, 2-naphthyl-α-ᴅ-glucopyranoside, 6-bromo-2-naphthyl-β-ᴅ-glucopyranoside, 1-naphthyl-*N*-acetyl-β-ᴅ-glucosaminide, 6-bromo-2-naphthyl-α-ᴅ-mannopyranoside, and 2-naphthyl-α-ʟ-fucopyranoside.

[d]Positive according to Palleroni (1984).

[e]Negative according to Tamaoka et al. (1987).

[f]Negative according to Palleroni (1984).

[g]Positive according to Tamaoka et al. (1987).

[h]11–89% of strains are positive (d score) according to Palleroni (1984).

[i]11–89% of strains are positive (d score) according to Tamaoka et al. (1987).

the chapter. The description below is taken from the original description (Gumaelius et al., 2001).

Cells are straight to slightly curved rods, 1–2 × 2–6 μm. On nutrient agar plates, yellow-white colonies are formed, and strain P17 produces a brownish pigment. On this medium cells occur singly or as filaments, motile by means of polar flagella. Growth at 20, 30, and 37°C, but not at 4°C. Reduces nitrate to nitrogen gas and is the only *Comamonas* species to do so. Contains cd₁-type nitrate reductase. All strains utilize fumarate, L-malonate, pyruvate, glycolate, D-β-hydroxybutyrate, α-ketovalerate, L-lactate, L-glutamate, L-lysine, D-saccharate, salicin, L-tartrate, D-glucuronate, succinate, L-alanine, and L-arginine. The following substrates are used by some of the four strains only: citrate, urea, *m*-hydroxybenzoate, *trans*-aconitate, maleinate, D-tartrate, gentisate, *p*-coumarate, hippurate, DL-2-γ-aminobutyrate, L-serine, and esculin. None of the strains use mannoic acid γ-lactone, L-arabinose, D-xylose, D-galactose, maltose, D-cellobiose, D-trehalose, palatinose, sucrose, D-lactose, melibiose, lactulose, β-gentiobiose, D-melezitose, L-raffinose, inosine, adonitol, *meso*-inositol, D-arabitol, glycerol, maltitol, D-sorbitol, dulcitol, L-sorbose, 2-deoxy-D-ribose, L-rhamnose, D-fucose, L-fucose, D-tagatose, D-amygdalin, arbutin, methyl-β-D-galactopyranoside, 5-keto-D-gluconate, D-gluconate, 6-*O*-α-D-galactopyranosyl-D-gluconic acid, D-galactonic acid γ-lactone, D-ribose, L-xylose, D-glucose, D-mannose, L-arabitol, *meso*-erythritol, D-mannitol, xylitol, D-fructose, 6-deoxy-D-galactose, 2′-deoxyinosine, inulin, methyl-α-D-mannopyranoside, methyl-α-D-xylopyranoside, methyl-α-D-galactopyranoside, starch, D-galacturonate, D-arabinose, D-turanose, D-glucuronolactone, glycogen, D-lyxose, N-acetyl-D-glucosamine, maltose, D-gluconate, caprate, adipate, maleate, phenylacetate, gelatin, p-nitrophenyl-β-D-galactopyranoside, L-tryptophan, L-histidine, L-ornithine, and arabic acid. Able to grow in 2% saline solution and to survive in 5% NaCl, but not in 9% NaCl. All strains are sensitive to chloramphenicol (30 μg), erythromycin (15 μg), streptomycin (30 μg), tetracycline (30 μg), and ampicillin (10 μg). Sensitivity to rifampicin (5 μg), sulfisoxazole (250 μg), and penicillin G (10 μg) varies among strains.

Isolated from activated sludge with biological nitrogen removal properties.

The mol% G + C of the DNA is: 60.4–60.8 (HPLC).

Type strain: 123, ATCC 700936, CCUG 44425.

GenBank accession number (16S rRNA): AF233877.

3. **Comamonas koreensis** Chang, Han, Chun, Lee, Rhee, Kim and Bae 2002, 380^VP

ko.re.en′ sis. N.L. fem. adj. *koreensis* of Korea, the geographical origin of isolation.

The characteristics are the same as those of the genus. The species is not included in Tables BXII.β.44 and BXII.β.45 because it was published after the completion of the chapter. The description below is taken from the original description (Chang et al., 2002).

Grows at 10, 25, and 37°C, but not at 5 or 42°C. Best growth at pH 7 and 30°C. Grows on nutrient agar with 3% (w/v) NaCl, but not 4.5 or 6.5% NaCl. Aerobic, but also able to grow anaerobically in an atmosphere of $H_2/CO_2/N_2$ (7:5:88) on nutrient agar. Nonmotile, and no flagella are produced. Nitrate is reduced to nitrite; no denitrification. Arginine dihydrolase, lysine decarboxylase, ornithine de-

carboxylase, and urease are not produced. The following substrates are utilized: cellobiose, malate, maltose, D-psicose, D-raffinose, L-rhamnose, D-glucose, aconitate, D-tagatose, *m*-inositol, and D-mannitol. The following substrates are not used: citrate, caprate, N-acetyl-D-galactosamine, N-acetylglucosamine, adonitol, esculin, amygdalin, DL-arabinose, DL-arabitol, arbutin, dulcitol, erythritol, DL-fucose, galactose, α-gentiobiose, glycogen, inulin, 2-ketogluconate, 5-ketogluconate, lactose, lactulose, D-lyxose, D-mannose, melibiose, melezitose, methyl-β-D-glucoside, methyl-α-D-glucoside, methyl-α-D-mannoside, methyl-β-D-xyloside, ribose, salicin, sorbitol, sorbose, sucrose, trehalose, D-turanose, DL-xylose, xylitol, adipate, D-gluconate, glycerol, propionate, sebacate, L-threonine, D-alanine, L-alanine, L-aspartate, L-leucine, L-histidine, L-phenylalanine, phenylacetate, D-fructose or starch. Hydrolyzes Tween 80, acetic acid, D-galactonic acid lactone, D-gluconic acid, itaconic acid, methyl pyruvate, monomethyl succinate, quinic acid, D-saccharic acid, succinamic acid, succinic acid, bromosuccinic acid, L-leucyl-2-naphthylamide, 2-naphthylcaprylate, 2-naphthyl phosphate (pH 5.4 and 8.5), and napthol-AS-BI phosphate. Does not hydrolyze gelatin, 2-naphthylbutyrate, 2-naphthyl phosphate (pH 8.5), alaninamide, L-alanylglycine, γ-aminobutyric acid, 2-aminoethanol, arginine, L-asparagine, DL-carnitine, glucuronamide, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, hydroxy-L-proline, phenylethylamine, L-proline, putrescine, L-pyroglutamic acid, DL-serine, citric acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, formic acid, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, inosine, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, thymidine, Tween 40, urocanic acid, uridine, 2,3-butanediol, α-cyclodextrin, dextrin, gelatin, DL-α-glycerol phosphate, glucose-1-phosphate, glucose-6-phosphate, N-benzoyl-DL-arginine-2-naphthylamide, 6-bromo-2-naphthyl-α-D-galactopyranoside, 6-bromo-2-naphthyl-β-D-glucopyranoside, 6-bromo-2-naphthyl-α-D-mannospyranoside, L-cystyl-2-naphthylamide, N-glutaryl-phenylalanine-2-naphthylamide, 2-naphthylmyristate, 2-naphthyl-β-D-galactopyranoside, naphthol-AS-BI β-D-glucuronate, 2-naphthyl-α-D-glucopyranoside, 1-napthyl-N-acetyl β-D-glucosaminide, 2-naphthyl α-L-fucopyranoside, and L-valyl-2-naphthylamide. Acetoin, H_2S, and indole are not produced. No β-galactosidase and tryptophan deaminase.

Major fatty acids are hexadecanoic acid ($C_{16:0}$), *cis*-9-hexadecanoic acid ($C_{16:1\ \omega7c}$), methylene-hexadecanoic acid ($C_{17:0\ cyclo}$), and octadecanoic acid ($C_{18:1}$); 2-hydroxy fatty acids ($C_{15:0\ 2OH}$ and $C_{16:0\ 2OH}$) and 3-hydroxy fatty acids ($C_{10:0\ 3OH}$) are present in smaller amounts (<4 %).

Isolated from a wetland sample in Woopo, Republic of Korea.

The mol% G + C of the DNA is: 66 (T_m).

Type strain: YH12, KCTC 12005, IMSNU 11158.

GenBank accession number (16S rRNA): AF275377.

4. **Comamonas nitrativorans** Etchebehere, Errazquin, Dabert, Moletta and Muxí 2001b, 982^VP

ni.tra.ti.vo′ rans. N.L. n.*nitras* nitrate; L. adj. part. *vorans* devouring, digesting; N.L. adj. *nitrativorans* nitrate-consuming.

The characteristics are the same as those of the genus. The species is not included in Tables BXII.β.44 and

BXII.β.45 because it was published after the completion of the chapter. The description below is taken from the original description (Etchebehere et al., 2001b).

Cells are motile with two tufts of polar flagella. On TSA colonies are cream-colored, circular, and 1–2 mm in diameter after 24 h. Growth on acetate, butyrate, n-caproate, i-butyrate, i-valerate, propionate, n-valerate, lactate, alanine, benzoate, L-phenylalanine, and ethanol. Weak growth on maleate. No growth on glucose, arabinose, fructose, galactose, xylose, mannitol, malonate, tartrate, p-aminobenzoate, gluconate, pyruvate or citrate. Anoxic reduction of nitrate, nitrite, and nitrous oxide to nitrogen. Under anaerobic conditions, the same substrates (except benzoate and phenylalanine) can be used as under aerobic conditions. Optimal pH is 7, and optimal temperature for growth is 30°C.

Isolated from a denitrifying reactor from a landfill leachate treatment system in Montevideo, Uruguay.

The mol% G + C of the DNA is: unknown.
Type strain: 23310, CIP 107121, DSM 13191, NCCB 100007, CCT 7062.
GenBank accession number (16S rRNA): AJ251577.

5. **Comamonas testosteroni** (Marcus and Talalay 1956) Tamaoka, Ha, and Komagata 1987, 58[VP] emend. Willems, Pot, Falsen, Vandamme, Gillis, Kersters and De Ley 1991c, 440 (*Pseudomonas testosteroni* Marcus and Talalay 1956, 661.)

tes.tos.te.ro' ni. M.L. gen. n. *testosteroni* of testosterone, a chemical compound.

The characteristics are as described for the genus and as indicated in Tables BXII.β.44 and BXII.β.45.

Isolated from soil.

The mol% G + C of the DNA is: 62.5–64.5 (species); 62.5 (type strain) (T_m).
Type strain: ATCC 11996, LMG 1786, NCTC 10698.
GenBank accession number (16S rRNA): M11224.

Genus II. **Acidovorax** Willems, Falsen, Pot, Jantzen, Hoste, Vandamme, Gillis, Kersters and De Ley 1990, 394[VP] emend. Willems, Goor, Thielemans, Gillis, Kersters and De Ley 1992a, 115

ANNE WILLEMS AND MONIQUE GILLIS

A.ci.do.vo' rax. L. neut. n. *acidum* acid; L. adj. *vorax* voracious; M.L. masc. n. *Acidovorax* acid-devouring (bacteria).

Straight to slightly curved rods, 0.2–1.2 × 0.8–5.0 µm, occurring singly, in pairs, or in short chains. **Gram negative. Motile by means of one or rarely two or three polar flagella. Aerobic, having a strictly oxidative type of metabolism with O_2 as the terminal electron acceptor**; some strains of two species (*Acidovorax delafieldii* and *Acidovorax temperans*) are capable of **heterotrophic denitrification** of nitrate. Most strains do not produce pigments on nutrient agar, but some phytopathogenic strains may produce a yellow to slightly brown diffusible pigment. **Oxidase positive; urease activity varies among strains. Chemoorganotrophic**, although strains of two species (*A. facilis* and *A. delafieldii*) can grow **lithoautotrophically**, using the oxidation of H_2 as an energy source. Good growth occurs on organic acids, amino acids, and peptone, but organisms show less versatile growth on carbohydrates. Fatty acids present always include 3-hydroxyoctanoic acid ($C_{8:0\ 3OH}$) and 3-hydroxydecanoic acid ($C_{10:0\ 3OH}$); 2-hydroxy-substituted fatty acids are absent. *Acidovorax* strains can be isolated from soil, water, clinical samples, activated sludge, and infected plants.

The mol% G + C of the DNA is: 62–70.

Type species: **Acidovorax facilis** (Schatz and Bovell 1952) Willems, Falsen, Pot, Jantzen, Hoste, Vandamme, Gillis, Kersters and De Ley 1990, 394 (*Pseudomonas facilis* (Schatz and Bovell 1952) Davis, Doudoroff, Stanier and Mandel 1969, 385; "*Hydrogenomonas facilis*" Schatz and Bovell 1952, 88.)

FURTHER DESCRIPTIVE INFORMATION

Flagellation and pili *Acidovorax* cells have one or, rarely, two to three polar flagella (Willems et al., 1992a). In a study of the cell morphology and flagellation of Gram-negative hydrogen bacteria, Aragno et al. (1977) defined three types of flagella on the basis of fine structural details as revealed by electron microscopy. They have reported *A. facilis* to possess a polar monotrichous flagellation, with flagella of 19–20 nm in diameter, a wavelength of 1.4–1.7 mm, and a fine structure of type I. In *A. facilis*, pili may be spread over the total cell surface, but they are observed only rarely (Aragno et al., 1977).

Cell wall composition In a study of the cell envelope of Gram-negative hydrogen bacteria, Walther-Mauruschat et al. (1977) distinguished three types of cell walls, differing mainly in the visibility and location of the peptidoglycan layer. *Acidovorax* possesses type I cell walls, typical of most Gram-negative bacteria and characterized by a multilayered structure, consisting of an outer membrane and a cytoplasmic membrane of similar dimensions and appearance separated by a dense layer of peptidoglycan (Walther-Mauruschat et al., 1977). *A. delafieldii* exhibits a crystalline S layer in close contact with the outer membrane and covering the whole cell surface (Lapchine, 1979; Chalcroft et al., 1986). A second major type of outer membrane protein, Omp34, has been purified and characterized as a typical porin, forming anion-selective channels. The channel conductance depends on ion concentration (Brunen et al., 1991). Functional properties of Omp34 have been studied and are largely determined by positively charged amino acid residues (Brunen and Engelhardt, 1995). The finding of anion-selective porins is compatible with the preference of *Acidovorax* strains for acidic substrates.

Fine structure Intracellular mesosome-like membrane systems with a spiral appearance, often located in the area of cell division or at the cell poles, have been reported in *A. facilis* (Walther-Mauruschat et al., 1977). However, there is a growing consensus that such structures are mostly artifacts resulting from the preparation of cells for electron microscopy. Intracellular inclusions of poly-β-hydroxybutyrate and polyphosphate and translucent glycogen-like inclusions have been detected in *A. facilis* (Walther-Mauruschat et al., 1977). *A. avenae* has been re-

ported to contain inclusion bodies consisting of a proteinaceous ribbon, which is rolled up to form a hollow cylinder (Wells and Horne, 1983). The function of these so-called R-bodies remains uncertain. Similar structures have been described in some kappa-particles, the endosymbionts in killer paramecia, where they are thought to be involved in toxin release (Lalucat et al., 1982).

Colony morphology On nutrient agar, colonies are round with smooth to slightly scalloped or spreading margins. Occasionally, two different but unstable colony margin types may be observed in a culture. A translucent marginal zone may be present. Colonies are convex, smooth to slightly granular, and beige to faintly yellow. At 30°C, colonies can attain diameters of 0.5–3 mm in 3 d and 4 mm in 7 d. Most species do not produce pigments on nutrient agar, but some phytopathogenic strains may produce a yellow to slightly brown diffusible pigment (Willems et al., 1992a; Gardan et al., 2000). Optimal growth temperature is 30–35°C.

Nutrition and metabolism A variety of organic compounds can be used as sole carbon sources (Tables BXII.β.46 and BXII.β.47). *A. facilis* and some strains of *A. delafieldii* are able to grow lithoautotrophically, using the oxidation of hydrogen as an energy source. The hydrogenase is membrane-bound and does not reduce NAD (Schneider and Schlegel, 1977).

Chemotaxonomic characteristics *A. facilis*, *A. delafieldii*, and *A. avenae* have been reported to contain putrescine and 2-hydroxyputrescine as major polyamines and spermidine and spermine in smaller quantities. Some strains contain traces to small amounts of cadaverine and 1,3-diaminopropane (Busse and Auling, 1988; Auling et al., 1991). The fatty acid contents of the four nonphytopathogenic species *A. delafieldii*, *A. facilis*, *A. defluvii* and *A. temperans* have been determined (Willems et al., 1992a; Schulze et al., 1999a). For the phytopathogenic species *A. avenae* and *A. konjaci*, data are limited to five strains of *A. avenae* (Willems et al., 1990) and two strains of *A. anthurii* (Gardan et al., 2000). For all these species major fatty acids (representing at least 11% of total fatty acids) are palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), and *cis*-vaccenic acid ($C_{18:1\ \omega7c}$); small amounts (less than 6%) of lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), and 3-hydroxy-decanoic acid ($C_{10:0\ 3OH}$), and smaller amounts still (less than 1%) of *n*-pentadecanoic acid ($C_{15:0}$), *n*-heptadecanoic acid ($C_{17:0}$), oleic acid ($C_{18:1\ \omega7c}$), stearic acid ($C_{18:0}$), and cyclopropane-substituted methylene-hexadecanoic acid ($C_{17:0\ cyclo}$) are present. In addition, the nonphytopathogenic species contain 1–2.5% 3-hydroxyoctanoic acid ($C_{8:0\ 3OH}$). No 2-hydroxy substituted fatty acids are present (Willems et al., 1990).

Plasmids Mitomycin treatment of *A. delafieldii* cells results in high numbers of mutants unable to oxidize hydrogen, suggesting that in this species, hydrogenase may be plasmid encoded (Pootjes, 1977); the species has been reported to contain a plasmid (Gerstenberg et al., 1982). However, mutants of *A. facilis* defective in lithoautotrophy, obtained by Tn5 mutagenesis, mitomycin C treatment, and incubation at sublethal temperature, all show wild-type plasmid patterns with the same restriction map. Preliminary hybridization experiments have revealed a transposon location in the chromosomal DNA and not in the plasmid (Warrelmann and Friedrich, 1986).

In a study of the influence of earthworm activity on the transfer of plasmid pJP4 from an inoculated *Pseudomonas fluorescens* strain to indigenous soil bacteria, *Acidovorax* species have been identified among transconjugants (Daane et al., 1996).

A highly efficient transformation system using pBR322-derived plasmid vectors with *A. avenae* as a host bacterium has been developed, following observations of efficient transformation of plasmid pBR322 and derived plasmids in *A. avenae* strain K1 and its proline-auxotrophic mutant Pr47 (Fukumoto et al., 1997).

Phages Phages have been reported in *Acidovorax* only in the original description of *"Hydrogenomonas facilis"*, where the occurrence of plaques in autotrophic cultures was observed approximately two months after isolation (Schatz and Bovell, 1952). Repeated streaking on mineral agar eliminated the plaques, but they reappeared six months later and again a year after purification. Plaques were 3–4 mm in diameter and contained numerous small resistant colonies. It was suggested that the abundance and rapid growth of these resistant strains explained why liquid cultures never cleared. Phages were never observed in heterotrophic cultures (Schatz and Bovell, 1952).

Ecology *A. delafieldii* and *A. facilis* constitute the dominant microorganisms from soils, sludge, old compost, and freshwater sources that are able to degrade poly-3-hydroxybutyrate (PHB) and poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) *in vitro* (Mergaert and Swings, 1996). *A. avenae* subsp. *avenae* is able to depolymerize synthetic thermoplastic polymers, such as poly-butylene succinate-co-butylene adipate and poly-caprolactone and poly-caprolactone-starch composites, and has been successfully applied in an *in vitro* biodegradation test system (Scandola et al., 1998). Elevated copper concentrations inhibit the degradation of powdered PHB by *A. delafieldii*, as demonstrated using an overlay agar technique (Birch and Brandl, 1996). *A. delafieldii* biofilms have been shown to contribute to copper solvency in laboratory reactors, suggesting that they may contribute to copper dissolution and increased bulk phase copper levels in domestic water systems (Davidson et al., 1996).

Antibacterial and antifungal activities may give pathogenic bacteria an advantage over competitors and, therefore, such activities of phytopathogenic *Acidovorax* species have been investigated. Effects against various Gram-negative and Gram-positive bacteria have been tested, but only *A. avenae* subsp. *cattleyae* shows antibacterial activity, and this is limited to just one of the bacteria tested, *Listeria innocua*. When antifungal activity was tested against *Rhodotorula mucilaginosa*, most strains of *A. avenae* subsp. *avenae* and *A. avenae* subsp. *cattleyae* showed clear antifungal activity, *A. avenae* subsp. *citrulli* strains showed weak or variable antifungal activity, and *A. konjaci* showed no antifungal activity (Hu and Young, 1998).

Pathogenicity Three *Acidovorax* species are the causal agents of diseases on various plants. *A. avenae* subsp. *avenae* causes symptoms of leaf blight in many members of the Poaceae family, including corn (*Zea mays*), sugar cane (*Saccharum officinarum*), and rice (*Oryza sativa*) (Claflin et al., 1989). Small round to oval-shaped, grayish white spots, with a red margin occur and may merge to form red longitudinal stripes. Sometimes, water-soaked lesions occur, which may develop into large necrotic zones. Stalk rot and bud rot have also been reported (Manns, 1909; Rosen, 1922; Lee et al., 1925; Okabe, 1934; Hayward, 1962; Baraoidan, 1981). *A. avenae* subsp. *cattleyae* causes brown spot disease on *Cattleya* and *Phalaenopsis* orchids. Large water-soaked lesions occur on leaves and quickly turn brown, with the surrounding tissue showing yellow-green halos. Large necrotic spots result, and the plant may die if the growth tip is affected (Ark and Thomas, 1946). *A. avenae* subsp. *citrulli* is pathogenic for watermelon (*Ci-*

TABLE BXII.β.46. Characteristics differentiating the species of the genus *Acidovorax*[a]

Characteristics	A. facilis	A. avenae subsp. avenae	A. avenae subsp. cattleyae	A. avenae subsp. citrulli	A. delafieldii	A. konjaci	A. temperans
Growth in the presence of 3% NaCl	−				−	+	−
Peptonization of milk		−	−	d		+	
Soluble starch utilization	−	d	+	d[b]	−	−	−
Hydrolysis of Tween 80	+	+		+	d	+	−
Hydrolysis of gelatin	+	−	−	d	d	−	−
Nitrite reduction	−	d		d	d	+	+
Autotrophic growth with H_2	+				d		−
Production of H_2S	−		−		−	+	−
Growth on:							
D-Glucose	+	+	+	+[b]	+	−	+
D-Mannose	+	−	−	−	+	−	−
L-Arabinose	+	+	+	+[b]	+	−	−
D-Ribose	+	d	+	+	+	+	−
D-Xylose	−	+	−	d[c,d]	−	−	−
D-Galactose	+	+	+	+	+	−	−
D-Mannitol	+	+	+	−	+	+	d
D-Arabitol	+	+	+	−	+	+	d
Sorbitol	+	+	+	−[e]	+	−	d
D-Fucose	−	+[f]	+	d	+[c]	−	−
Dextrin		+		−		−	
2-Ketogluconate	−	−	−	d	+	−	d
L-Threonine, L-histidine	+	+	+	−	+	+	d
L-Tryptophan	+[c]	+	+	d	d	+	−
Ethanolamine	−	d	+	+[e]	−	−[e]	−
DL-2-Aminobutyrate	−	d	+	−	−	−	d
DL-3-Aminobutyrate	−	d	+	d	−	+	−
2-Aminopentanoate		+		+		−	
Acetamide	−	d	+	−	−	−	−
Isobutyrate	−	+	+	d	d	+	d
Isovalerate	+[c]	+	+	−	+	+	+
n-Caproate	−	d	+	−	d	−	d
Adipate	−	+	+	+	+	+	+
D-Tartrate	−	+	+	+[g]	d	+[g]	−
m-Tartrate	−	d	−	+[e,h]	+	+	d
2-Ketoglutarate	−	+	+	+	+[c]	+	d
Citraconate	−	+	+	+	+	+	d
Aconitate	−	d	−	−	d	+	d
Citrate	−	d[h]	+	d[h,i]	d	d[c,j]	−
Butane-2,3-diol		+		d	−		
p-Hydroxybenzoate	d	−	−	−	+	−	d
Anthranilate		+		d		−	
Acid from:							
D-Glucose	−	+	+		−		−
D-Fructose	−		+		−	+	−
D-Xylose	−		+		−		−
Mol% G + C of DNA	64–65	68–70	69	67–68	65–66	68	62–66

[a]for symbols see standard definitions. Data taken from Haynes and Burkholder (1957) (for *A. avenae* subsp. *cattleyae*), Schaad et al. (1978), Goto (1983), Ramundo and Claflin (1990), Willems et al. (1990, 1992a), and Hu et al. (1991).

[b]Negative according to Schaad et al. (1978).

[c]Late positive reaction (5 days).

[d]Positive according to Hu et al. (1991).

[e]Eleven to 89% of strains are positive (d score) according to Hu et al. (1991).

[f]The type strain is negative.

[g]Negative according to Hu et al. (1991).

[h]Weak growth.

[i]Positive according to Schaad et al. (1978).

[j]Positive according to Goto (1983).

trullus lanatus) and other members of the family Cucurbitaceae, in which it causes bacterial fruit blotch. The disease appears first on the upper side of the fruit, where dark olive green stains rapidly increase in size. The rind ruptures, allowing access to organisms causing secondary rotting and ultimately leading to the collapse of the fruit. Leaf lesions are inconspicuous, small, dark brown spots with margins that, when viewed from the bottom of the leaf, appear water-soaked (Latin and Hopkins, 1995).

A. konjaci causes leaf blight on *Amorphophallus rivieri* cv. Konjac, a food crop in Japan. It is characterized by small water-soaked spots, which soon extend to become larger lesions with yellowish halos. The whole leaf may ultimately rot away and drop, leaving just bare stalks (Goto, 1983). *A. anthurii* causes leaf spot on *Anthurium*; first symptoms consist of necrotic lesions close to veins and leaf margins, which blacken and turn gray (Gardan et al., 2000). From these lesions the bacteria enter the leaf and spathe

TABLE BXII.β.47. Other characteristics of the species of the genus *Acidovorax*[a]

Characteristics	A. *facilis*	a. A. *avenae* subsp. *avenae*	b. A. *avenae* subsp. *cattleyae*	c. A. *avenae* subsp. *citrulli*	A. *delafieldii*	A. *konjaci*	A. *temperans*
Growth at:							
4°C		−	−	−		−	
30°C	+		+		+		+
37°C	d				+		+
41°C		d		d[b]		−[c]	
42°C	−				d		d
Growth in the presence of:							
0.5% NaCl	+				+		+
1.5% NaCl	d				d		d
4.5 and 6.5 % NaCl	−				−		−
Growth on Drigalski–Conradi agar	−				d		d
Urease	d	+		d	d	+	d
Catalase	+				d	+	d
ONPG (β-Galactosidase)	−				−		−
Lysine and ornithine decarboxylase	−				−	−	−
Arginine dihydrolase	−	−		−	−	d[d]	−
Phenylalanine deaminase, arginine decarboxylase						+	
Hemolysis (α and β)	−				−		−
Alkaline reaction in milk		−	−	−[b]		−	
Acid reaction in milk		−	−	−		−	
Potato starch utilization		d		d		d	
Hydrolysis of DNA, esculin, acetamide	−				−		−
Pectate lyase		d		d		−	
Polygalacturonase		−		−		−	
Nitrate reduction	+	d	+	+[e]	+	+	+
Denitrification	−				d	−	d
Acid production in 10% lactose and in triple sugar iron medium	−				−		−
Production of 2-ketogluconate				+			
Levan production		−		−			
Fluorescence	−			−	−		−
Growth on cetrimide	−				−		−
Indole production	−		−	−	−	−	−
Resistance to penicillin (10 μg/disk)	d				d		d
Growth on:[f]							
Xylan, glucitol, *meso*-erythritol, *myo*-inositol, ribitol, galactitol, carboxymethyl cellulose						−	
D-Melibiose		−[g]		−[h]	−	−[h]	−
L-Rhamnose, lactose, D-cellobiose, trehalose, salicin, inuline, erythritol	−	−[g]	−	−	−	−	−
Maltose	−	−	−	−[g]	−	−	−
Sucrose	−	−[g]	−	−[g]	−	−[g]	−
D-Raffinose, D-melizitose	−	−	−	−	−	−[g]	−
L-Fucose	−	d	−	−	−	−	−
Gluconate	+	+	d	+	+	+[d]	d
D-Lactate	d	+		+	+	+	+
D-Lactate plus methionine	d				+		+
D-α-Alanine, L-norleucine	d	+	+	+	d	+	d
L-α-Alanine, L-leucine, L-isoleucine, L-serine, L-phenylalanine, L-tyrosine, L-aspartate, β-alanine	+	+	+	+	+	+	d
L-Cysteine	−	−	−	d	−	−	−
DL-Norleucine	−				d		d
L-Valine	+	+	+	d	d	+	d
DL-Norvaline	−	d	d	−	−	−	d
D-Serine				+			
L-Arginine	−	−	−	−	−	d	−
L-Methionine	d[i]	−	−	−	−	−	d
L-Ornithine	d[i]	d	+	d	d	d	d
L-Citrulline	−	−	−	−	d	−	d
DL-Kynurenine, DL-5-aminovalerate	−	d	−	−	−	−	−
DL-4-Aminobutyrate	+	+	+	+	+	+	d
2-Aminopentanoate		+		+		−	
Formate, hippurate						−	
Acetate	d	+	+	+	d	d[c,i]	+
Propionate	+[i]	+	+	d[i]	d	+[g]	d
Butyrate, sebacate	+	+	+	+	+	+[d]	+
n-Valerate	+	+	+	d	+	+	+
Caprate	−	d	−	−	−	−[c]	−
Oxalate	−	−[g]	−	d[b,i]	−	d[d,i,j]	−
Succinate	+	+[g]	+	+	+	+[g]	+

(continued)

TABLE BXII.β.47. *(cont.)*

Characteristics	*A. facilis*	*A. avenae* subsp. *avenae*	*A. avenae* subsp. *cattleyae*	*A. avenae* subsp. *citrulli*	*A. delafieldii*	*A. konjaci*	*A. temperans*
Malonate	−	−[g]	−	−	−	+[g]	−
Heptanoate	−	−	−	−	−	−	d
Maleate	−	−	−	d[i]	d	−[c]	d
Glutarate	d	+	+	+	+	+	d
Pimelate	d	+	+	+	+	+	+
DL-Glycerate	+	+	+	+	+	+	d
L-Tartrate	−	−[g]	−	−	−	d[h]	−
Pyruvate	+	+	+	+	d	+	d
Mesaconate	−	−	−	−	−	d[i]	−
Levulinate	d	+	+	+	d	+[g]	d
Ethanol, propanol					+	+	
m-Hydroxybenzoate, phthalate	−	−	−	−	d	−	−
Acid from:							
Maltose, adonitol	−				−		−
D-Ribose, ethanol						+	
Glycerol		+	+			+	
D-Mannitol			+			+	
Galactose, arabinose, lactose, sucrose, dulcitol			+				
Raffinose		−	−				
myo-Inositol, rhamnose		−					
Hydrolysis of:							
2-Naphthylbutyrate, 2-naphthylcaprylate, L-leucyl-2-napthylamide	+					+	+
2-Naphthylphosphate (pH 8.5)	d				d		d
2-Naphthylphosphate (pH 5.4)	d				−		−
2-Naphthylmyristate	d				d		d
L-Cystyl-2-naphthylamide	−				d		d

[a]For symbols see standard definitions. Data taken from Haynes and Burkholder (1957) (for *A. avenae* subsp. *cattleyae*), Schaad et al. (1978), Goto (1983), Ramundo and Claflin (1990), Willems et al. (1990, 1992a), and Hu et al. (1991).

[b]Positive according to Schaad et al. (1978).

[c]Positive according to Goto (1983).

[d]Negative according to Goto (1983).

[e]Negative according to Schaad et al. (1978).

[f]Growth on the following substrates was positive for all *Acidovorax* species: D-fructose, glycerol, D-malate, L-malate, fumarate, suberate, azelate, DL-lactate, DL-3-hydroxybutyrate, L-glutamate, and L-proline. Growth on the following substrates was negative for all *Acidovorax* species: D-arabinose, L-xylose, adonitol, D-lyxose, L-arabitol, methyl-xyloside, L-sorbose, D-tagatose, dulcitol, inositol, methyl-D-mannoside, methyl-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, β-gentiobiose, D-turanose, starch, glycogen, xylitol, 5-ketogluconate, L-mandelate, caprylate, pelargonate, glycolate, itaconate, phenylacetate, benzoate, *o*-hydroxybenzoate, D-mandelate, isophthalate, ter-ephthalate, glycine, D-tryptophan, trigonelline, L-lysine, betaine, creatine, 3-aminobenzoate, 4-aminobenzoate, urea, sarcosine, ethylamine, butylamine, amylamine, ben-zylamine, diaminobutane, spermine, histamine, tryptamine, and glucosamine. In addition, *A. facilis*, *A. delafieldii*, and *A. temperans* do not hydrolyze the following substrates: L-valyl-2-naphthylamide, *N*-benzoyl-DL-arginine-2-naphthylamide, *N*-glutaryl-phenylalanine-2-naphthylamide, 6-bromo-2-phosphodiamide-3-naphthoic acid-2-methoxyanilide (= napthol-AS-BI-phosphodiamide), 6-bromo-2-naphthyl-α-D-galactopyranoside, 2-naphthyl-β-D-galactopyranoside, 6-bromo-2-hydroxy-3-naphthoic acid-2-methoxyanilide-β-D-glucuronate (= naphthol-AS-BI-β-D-glucuronate), 2-naphthyl-α-D-glucopyranoside, 6-bromo-2-naphthyl-β-D-glucopyranoside, 1-naphthyl-*N*-acetyl-β-d-glucosaminide, 6-bromo-2-naphthyl-α-D-mannopyranoside, and 2-naphthyl-α-L-fucopyranoside.

[g]11–89% of strains are positive (d score) according to Hu et al. (1991).

[h]Positive according to Hu et al. (1991).

[i]Late positive reaction (5 days).

[j]Negative according to Hu et al. (1991).

parenchymas and become systemic, resulting in tissue discolor-ation and plant death.

Pathogenicity tests with *A. avenae* subsp. *avenae* on water-melon, cantaloupe, squash, cucumber, tomato, and cowpea are negative (Schaad et al., 1978). Similar tests with *A. avenae* subsp. *citrulli* on tomato, cowpea, corn, and Konjac are also negative (Schaad et al., 1978; Goto, 1983). Tests with *A. konjaci* on canta-loupe, squash, and cucumber are negative and on watermelon results in a hypersensitive reaction (Goto, 1983). Phytopatho-genicity of the other *Acidovorax* species has not been reported.

ENRICHMENT AND ISOLATION PROCEDURES

For most *Acidovorax* species, no special isolation procedures have been described, and most strains have been isolated using stan-dard techniques from soil, water, sludge, or infected plant ma-terial. These normally result in the isolation of strains of various taxa, except for phytopathogenic species, which are isolated from infected tissue that often contains only the causal bacterium. *A. facilis* was originally isolated from lawn soil in a mineral medium[1] supplemented with 0.05% NaHCO$_3$ and incubated at 25°C under an atmosphere of CO$_2$/air/H$_2$ (10:30:60) (Schatz and Bovell, 1952). *A. delafieldii* was originally isolated from soil, using poly-3-hydroxybutyrate as the sole carbon source in a mineral liquid enrichment culture (Delafield et al., 1965). This procedure yielded strains belonging to various species of Gram-negative, polarly flagellated, straight to slightly curved, nonsporeforming rods, one of which was later named *Pseudomonas delafieldii* (Davis et al., 1970). A phenanthrene-degrading *A. delafieldii* strain has been isolated from creosote-contaminated soil by enrichment cultures on phenanthrene (Shuttleworth and Cerniglia, 1996).

1. The mineral medium contains (g/l distilled water) KH$_2$PO$_4$, 1.0; NH$_4$NO$_3$, 1.0; MgSO$_4$·7H$_2$O, 0.2; FeSO$_4$·7H$_2$O, 0.01; and CaCl$_2$·2H$_2$O, 0.01; pH 6.8–7.2 (Schatz and Bovell, 1952).

Aznar et al. (1992) have isolated *A. delafieldii* from a hypertrophic freshwater lagoon on casein peptone starch agar and have studied *A. delafieldii* strains isolated from healthy and diseased eels from eel farms. Some isolates of *A. delafieldii* and *A. temperans* have originated from various clinical sources, where they were isolated using routine clinical techniques (Willems et al., 1990). *A. defluvii* was isolated from activated sludge form a wastewater treatment plant in Munich (Schulze et al., 1999a).

The phytopathogenic species can be isolated from infected plant material. Small pieces (about 1 cm²) of diseased tissue are cut from lesions in leaf blades, surface-sterilized (for example, with 70% ethanol (Goto, 1983), 0.5% sodium hypochlorate (Schaad et al., 1978), or 70% ethanol followed by 0.001% mercuric chloride and again 70% ethanol (Hayward, 1962) and rinsed with sterile distilled water. The tissue may be further mashed mechanically. The suspension can then be plated out immediately or left to diffuse at room temperature for several hours before plating out on a suitable medium, such as yeast extract–peptone agar. Plates are incubated at 28–30°C, and colonies usually appear within 2 days (Hayward, 1962; Schaad et al., 1978; Goto, 1983).

MAINTENANCE PROCEDURES

Acidovorax strains can be maintained on nutrient agar at 4°C for up to 2 months. Unlike some autotrophic species, *A. facilis* does not readily lose its H_2-oxidizing capacity when grown heterotrophically over long periods of time or in the presence of 100% O_2 (Schatz and Bovell, 1952).

For long-term preservation, strains can be lyophilized using standard procedures. For example, at the BCCM-LMG (Ghent, Belgium) culture collection this is routinely done in horse serum.

DIFFERENTIATION OF THE GENUS *ACIDOVORAX* FROM OTHER GENERA

See Table BXII.β.42 for the family *Comamonadaceae* for features differentiating *Acidovorax* from the other genera of this family.

An oligonucleotide probe for the genus *Acidovorax*, which hybridizes to variable region II of the 16S rRNA gene, has been designed to allow differentiation from species of *Comamonas* and *Hydrogenophaga*, but hybridization conditions are crucial. In the presence of 0–10% formamide, *Acidovorax* strains, and *Variovorax paradoxus*, hybridize with the probe. In the presence of 20% formamide, *Variovorax paradoxus* no longer hybridizes with the probe, but *A. delafieldii* is also negative (Amann et al., 1996).

TAXONOMIC COMMENTS

Pseudomonas facilis was originally described as *"Hydrogenomonas facilis"*, a hydrogen oxidizing isolate from lawn soil (Schatz and Bovell, 1952). When the genus *"Hydrogenomonas"* was later abandoned, *"Hydrogenomonas facilis"* was transferred to the genus *Pseudomonas* (Davis et al., 1969). *Pseudomonas delafieldii* was proposed for a number of strains phenotypically similar to *Pseudomonas facilis* but unable to oxidize hydrogen (Davis et al., 1970). By DNA–rRNA hybridization, it was later shown that the genus *Pseudomonas* was polyphyletic and consisted of five distinct and remotely related groups that could not be maintained in a single genus (De Vos and De Ley, 1983). *Pseudomonas facilis* and *Pseudomonas delafieldii* belong to the acidovorans rRNA complex in the *Betaproteobacteria*. In a polyphasic study of the relationships within this group, it was shown that both species, together with a number of clinical isolates that had been provisionally grouped in the so-called EF groups 13 and 16, form a separate rRNA

subbranch, for which the genus *Acidovorax* was proposed. Initially, three species were proposed: *Acidovorax facilis* for *Pseudomonas facilis*, *Acidovorax delafieldii* for *Pseudomonas delafieldii* and most of the strains of EF group 13, and *Acidovorax temperans* for most of the strains from EF group 16 and several misnamed *Pseudomonas* and *Alcaligenes* strains (Willems et al., 1990).

In an extension of this polyphasic study, several phytopathogenic *Pseudomonas* species were also transferred to the genus *Acidovorax* (Willems et al., 1992a). Several species causing disease on Poaceae have been united in *Acidovorax avenae* subsp. *avenae*. The causal agent of leaf blight on oats in Ohio was first described by Manns (1909) as *Pseudomonas avenae*. A similar disease of foxtail in Arkansas was attributed to a new species *"Pseudomonas alboprecipitans"* (Rosen, 1922). Later, isolates from diseased corn from Georgia and Florida were compared with the descriptions of both species, and Schaad et al. (1975) concluded that *"Pseudomonas alboprecipitans"* was a later synonym of *Pseudomonas avenae*. *Pseudomonas rubrilineas*, the causal agent of red stripe disease on sugarcane, was originally described as *Phytomonas rubrilineans* (Lee et al., 1925), but was later transferred to *Xanthomonas* (Starr and Burkholder, 1942) and then to *Pseudomonas* (Hayward, 1962). Later Ramundo and Claflin (1990) demonstrated the synonymy between *Pseudomonas rubrilineas* and *Pseudomonas avenae* by comparing physiological, biochemical, and serological data. *"Pseudomonas setariae"*, the causal agent of brown stripe disease on *Setaria italica*, was first described as *"Bacterium setariae"* (Okabe, 1934), but was later transferred to *Pseudomonas* (Săvulescu, 1947). It was not included on the Approved Lists (Skerman et al., 1980) because the only extant strain was considered a member of *Pseudomonas avenae*.

A second subspecies, *Acidovorax avenae* subsp. *cattleyae*, has been proposed for a pathogen of *Cattleya* orchids (Willems et al., 1992a). This organism was first described as *"Bacterium cattleyae"* by Pavarino (1911); later, brown spot disease on *Cattleya* and *Phalaenopsis* species was attributed to *Phytomonas cattleyae* (Ark and Thomas, 1946). This species was later transferred to *Pseudomonas* (Săvulescu, 1947). Based on a phenotypic analysis and DNA–DNA hybridizations, Hu et al. (1991) considered *Pseudomonas cattleyae* to be a synonym of *Pseudomonas avenae*.

A third subspecies, *Acidovorax avenae* subsp. *citrulli*, has been proposed as the causal agent of leaf blight on watermelon. It had been described as *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, mainly because of its phenotypic similarity to the *Pseudomonas alcaligenes* group (Schaad et al., 1978). Hu et al. (1991) transferred this subspecies to *Pseudomonas avenae* as *Pseudomonas avenae* subsp. *citrulli*.

Acidovorax konjaci has been proposed as a separate species (Willems et al., 1992a) comprising *Pseudomonas pseudoalcaligenes* subsp. *konjaci*, a pathogen isolated from the leaves of the Konjac plant (*Amorphophallus konjac*) (Goto, 1983). In addition, this subspecies was first transferred as a separate subspecies to *Pseudomonas avenae* by Hu et al. (1991).

Thus, the genus *Acidovorax* now contains seven species, which can be separated in two groups: the soil and water inhabitants *A. facilis*, *A. delafieldii*, *A. defluvii*, and *A. temperans*, which also include some opportunistic clinical isolates, and the phytopathogenic species *A. avenae* with its three subspecies, *A. konjaci* (Willems et al., 1992a), and *A. anthurii* (Gardan et al., 2000). This separation into two groups is reflected in the 16S rDNA phylogeny of these organisms (Willems et al., 1992a), but they have been maintained in a single genus because of phenotypic similarities (Willems et al., 1992a). More recently, the 16S rRNA gene

sequence analysis of the type strains of the all *Acidovorax* species has confirmed that *Acidovorax* does form a separate phylogenetic cluster within the *Comamonadaceae*, with all species showing at least 95.5% sequence similarity, thus supporting the grouping of the previously described rRNA subbranches into a single genus. DNA–DNA hybridizations do not show any significant DNA-binding among any of the *Acidovorax* species. They demonstrate that *A. avenae* consists of at least three subgroups for which separate subspecies have been proposed (Willems et al., 1992a). DNA-binding values of 54–67% have been obtained among the subspecies, whereas within the subspecies, values of at least 75% are obtained. These three subspecies can also be distinguished by 16S rDNA sequence comparison (Wen et al., 1999).

List of species of the genus Acidovorax

1. **Acidovorax facilis** (Schatz and Bovell 1952) Willems, Falsen, Pot, Jantzen, Hoste, Vandamme, Gillis, Kersters and De Ley 1990, 394[VP] (*Pseudomonas facilis* (Schatz and Bovell 1952) Davis, Doudoroff, Stanier and Mandel 1969, 385; "*Hydrogenomonas facilis*" Schatz and Bovell 1952, 88.)
fa' ci.lis. L. adj. *facilis* ready, quick.

Wilde and Schlegel (1982) have reported strains to be tolerant to 60% O_2 when grown autotrophically and 100% O_2 when grown heterotrophically. Characteristics differentiating *A. facilis* from the other *Acidovorax* species are presented in Table BXII.β.46. Further descriptive information is provided in Table BXII.β.47. Isolated from lawn soil in the United States.

The mol% G + C of the DNA is: 64–65 (T_m); type strain, 65 (T_m).

Type strain: ATCC 11228, CCUG 2113, DSM 649, LMG 2193.

GenBank accession number (16S rRNA): AF078765.

2. **Acidovorax anthurii** Gardan, Dauga, Prior, Gillis and Saddler 2000, 245[VP]
an.thu' ri.i. L. fem. n. *Anthurium* anthurium; L. gen. n. *anthurii* of anthurium, referring to the plant from which the phytopathogenic bacterium was first isolated.

The characteristics are the same as those of the genus. This species is not included in Tables BXII.β.46 and BXII.β.47 because it was published after the completion of the chapter. The description below is taken from the original description (Gardan et al., 2000).

On YBGA, colonies are circular, raised with an entire margin. A white-creamy, and a brown diffusible pigment is produced.

Catalase and urease are positive. Poly-β-hydroxybutyrate is accumulated in the cell; arginine is used as the sole source of carbon. H_2S is produced from cysteine and cellulose is hydrolysed. Indole, levane, and acetoin are not produced. Casein and esculin are not hydrolyzed. Acid is produced from galactose, arabinose, and glycerol. Acetate, formate, glycerol, DL-5-aminobutyrate, D(−)tartrate, and azelate are utilized. Trehalose, caprylate, D-ribose, D-glucose, *N*-acetylglucosamine, L-arginine, saccharose, inositol, sarcosine, itaconate, D-xylose, L-tryptophan, esculin, and mannitol are not utilized. The sole hydroxylated fatty acid present is 3-hydroxy-decanoic acid.

All strains elicit a hypersensitive reaction on tobacco leaves (HR) and are pathogenic on anthurium, producing typical leaf-spot symptoms.

The mol% G + C of the DNA is: 63.5 (T_m).

Type strain: CFBP 3232, CIP 107058.

GenBank accession number (16S rRNA): AJ007013.

3. **Acidovorax avenae** (Manns 1909) Willems, Goor, Thielemans, Gillis, Kersters and De Ley 1992a, 115[VP] (*Pseudomonas avenae* Manns 1909, 1933; "*Pseudomonas alboprecipitans*" Rosen 1922, 383.)
a' ve.nae. M.L. n. *avena* genus of plants; M.L. gen. n. *avenae* of *Avena*.

This species contains only phytopathogenic strains, which are divided into three subspecies.

The mol% G + C of the DNA is: 67–70 (T_m).

a. **Acidovorax avenae** *subsp.* **avenae** (Manns 1909) Willems, Goor, Thielemans, Gillis, Kersters and De Ley 1992a, 117[VP] (*Pseudomonas avenae* Manns 1909, 1933; *Pseudomonas avenae* subsp. *avenae* Manns 1909, 1933; "*Pseudomonas alboprecipitans*" Rosen 1922, 383.)

Characteristics differentiating *A. avenae* subsp. *avenae* from the other *Acidovorax* species are presented in Table BXII.β.46. Further descriptive information is provided in Table BXII.β.47. This subspecies is pathogenic for various species of Poaceae, including oats, corn, wheat, barley, sorghum, rye, sugarcane, rice seedlings, Italian millet, pearl millet, barnyard millet, foxtail millet, finger millet, Proso millet, and Vasey grass. There are phytopathological indications that strains pathogenic to rice are different from strains pathogenic to other plants, supported also by the detection of a protein specific to the rice-pathogenic strains (Kadota et al., 1996).

The mol% G + C of the DNA is: 68–70 (T_m); type strain, 70 (T_m).

Type strain: ATCC 19860, LMG 2117, NCPPB 1011.

GenBank accession number (16S rRNA): AF078759.

Additional Remarks: The type strain was isolated from *Zea mays* in the United States in 1958.

b. **Acidovorax avenae** *subsp.* **cattleyae** (Pavarino 1911) Willems, Goor, Thielemans, Gillis, Kersters and De Ley 1992a, 118[VP] (*Pseudomonas avenae* subsp. *cattleyae* (Pavarino 1911) Hu, Young and Triggs 1991, 524; *Pseudomonas cattleyae* (Pavarino 1911) Săvulescu 1947, 11; "*Bacterium cattleyae*" Pavarino 1911, 234.)
catt' ley.ae. M.L. fem. n. *Cattleya* a genus of orchids; M.L. gen. n. *cattleyae* of *Cattleya*.

Characteristics differentiating *A. avenae* subsp. *cattleyae* from the other *Acidovorax* species are presented in Table BXII.β.46. Further descriptive information is provided in Table BXII.β.47. Causes leaf spot and bud rot on *Cattleya* and *Phalaenopsis* orchids.

The mol% G + C of the DNA is: 69 (T_m).

Type strain: ATCC 33619, LMG 2364, NCPPB 961.
GenBank accession number (16S rRNA): AF078762.

c. **Acidovorax avenae** *subsp.* **citrulli** (Schaad, Sowell, Goth, Colwell and Webb 1978) Willems, Goor, Thielemans, Gillis, Kersters and De Ley 1992a, 118^{VP} (*Pseudomonas avenae* subsp. *citrulli* (Schaad, Sowell, Goth, Colwell and Webb 1978) Hu, Young and Triggs 1991, 524; *Pseudomonas pseudoalcaligenes* subsp. *citrulli* Schaad, Sowell, Goth, Colwell and Webb 1978, 123.)

ci' trul.li. M.L. masc. n. *Citrullus* a genus in the cucumber family; M.L. gen. n. *citrulli* of *Citrullus*.

Characteristics differentiating *A. avenae* subsp. *citrulli* from the other *Acidovorax* species are presented in Table BXII.β.46. Further descriptive information is provided in Table BXII.β.47. Pathogenic for several species of Cucurbitaceae, including watermelon, cantaloupe, cucumber, and squash.

The mol% G + C of the DNA is: 67–68 (T_m); type strain, 68 (T_m).

Type strain: ATCC 29625, ICMP 7500, LMG 5376.
GenBank accession number (16S rRNA): AF078761.

Additional Remarks: The type strain was isolated from a water-soaked lesion on a cotyledon of *Citrullus lanatus* in the United States in 1977.

4. **Acidovorax delafieldii** (Davis *in* Davis, Stanier, Doudoroff and Mandel 1970) Willems, Falsen, Pot, Jantzen, Hoste, Vandamme, Gillis, Kersters and De Ley 1990, 396^{VP} (*Pseudomonas delafieldii* Davis *in* Davis, Stanier, Doudoroff and Mandel 1970, 12.)

de.la.fiel' di.i. M.L. gen. n. *delafieldii* of Delafield, named after F.P. Delafield, who first isolated this organism.

Characteristics differentiating *A. delafieldii* from the other *Acidovorax* species are presented in Table BXII.β.46. Further descriptive information is provided in Table BXII.β.47. Isolated from soil, water, and various samples from clinical origin.

The mol% G + C of the DNA is: 65–66 (T_m); type strain, 66 (T_m).

Type strain: ATCC 17505, DSM 64, LMG 5943.
GenBank accession number (16S rRNA): AF078764.

Additional Remarks: This strain was isolated from soil with PHB as the sole carbon source. Since it is not the most representative of the redefined species, strain CCUG 23830B (LMG 8909) has been proposed as an alternative reference strain for this species (Willems et al., 1990).

5. **Acidovorax defluvii** Schulze, Spring, Amann, Huber, Ludwig, Schleifer and Kämpfer 1999b, 1325^{VP} (Effective publication: Schulze, Spring, Amann, Huber, Ludwig, Schleifer and Kämpfer 1999a, 205.)

de.flu' vi.i. L. n. *defluvium* sewage; L. gen. n. *defluvii* of sewage.

The characteristics are the same as those of the genus. This species is not included in Tables BXII.β.46 and BXII.β.47 because it was published after the completion of the chapter. The description below is taken from the original description (Schulze et al., 1999a).

The following characteristics are positive for all strains: growth at 28°C, storage of polyhydroxybutyrate, and hydrolysis of L-alanine-pNA. The following characteristics are negative for all strains: growth at 37°C or in the presence of 1.5% NaCl. Utilization of the following substrates for growth: *N*-acetyl-glucosamine, L-arabinose, *p*-arbutin, D-cellulose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α-D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xylose, adonitol, *i*-inositol, D-mannitol, D-sorbitol, propionate, azelate, citrate, itaconate, mesaconate, β-alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan, *m*-hydroxybenzoate, *p*-hydroxybenzoate, and phenylacetate. Hydrolysis of esculin, pNP-β-D-galactopyranoside, pNP-α-D-glucopyranoside, and pNP-β-glucopyranoside.

The hydroxylated fatty acids 3-hydroxyoctanoic ($C_{8:0\ 3OH}$) and 3-hydroxydecanoic acid ($C_{10:0\ 3OH}$) are present. Isolated from activated sludge of a municipal wastewater treatment plant. The type strain is nonmotile under laboratory conditions, is catalase positive, and reduces nitrate.

The mol% G + C of the DNA is: 62–64 (T_m); value for the type strain, 62.

Type strain: BSB411, DSM12644, CIP 106824.
GenBank accession number (16S rRNA): Y18616.

6. **Acidovorax konjaci** (Goto 1983) Willems, Goor, Thielemans, Gillis, Kersters and De Ley 1992a, 118^{VP} *Pseudomonas avenae* subsp. *konjaci* (Goto 1983) Hu, Young and Triggs 1991, 524; *Pseudomonas pseudoalcaligenes* subsp. *konjaci* Goto 1983, 544.)

kon.ja' ci. M.L. gen. n. *konjaci* of the konjac plant, *Amorphophallus rivieri* cv. Konjac.

Characteristics differentiating *A. konjaci* from the other *Acidovorax* species are presented in Table BXII.β.46. Further descriptive information is provided in Table BXII.β.47. Causal agent of leaf blight of *Amorphophallus rivieri* cv. Konjac, a Japanese food crop.

The mol% G + C of the DNA is: 68 (T_m).

Type strain: ATCC 33996, ICMP 7733, LMG 5691.

GenBank accession number (16S rRNA): AF078760, AF137507.

Additional Remarks: The type strain was isolated from *Amorphophallus rivieri* cv. Konjac.

7. **Acidovorax temperans** Willems, Falsen, Pot, Jantzen, Hoste, Vandamme, Gillis, Kersters, and De Ley 1990, 396^{VP}

tem' pe.rans. L. v. *temperare* to moderate; M.L. pres. part. *temperans* moderate, referring to the moderate metabolic versatility of the species.

Characteristics differentiating *A. delafieldii* from the other *Acidovorax* species are presented in Table BXII.β.46. Further descriptive information is provided in Table BXII.β.47. Isolated from various samples from clinical environments; one strain was isolated from active sludge from a waste water purification plant in Sweden.

The mol% G + C of the DNA is: 62–66 (T_m); type strain 62 (T_m).

Type strain: CCUG 11779, DSM 7270, LMG 7169.
GenBank accession number (16S rRNA): AF078766.

Additional Remarks: The type strain was isolated from a urine sample of a 68-year-old male patient in Göteborg, Sweden.

Genus III. **Brachymonas** Hiraishi, Shin and Sugiyama 1995b, 879[VP] (Effective publication: Hiraishi, Shin and Sugiyama 1995c, 110)

AKIRA HIRAISHI

Bra.chy.mo'nas. Gr. adj. *brachy* short; Gr. n. *monas* a unit; M.L. fem. n. *Brachymonas* a small short unit.

Cells are coccobacilli or short rods, 0.6–1.0 × 0.8–1.5 µm, with rounded ends, occurring singly, in pairs, and in chains. Nonsporeforming, nonencapsulated, and nonmotile. Gram negative.

Aerobic chemoorganotrophs having a strictly respiratory type of metabolism, with oxygen as the terminal electron acceptor. **Growth occurs anaerobically with nitrate** as the terminal electron acceptor. Denitrification positive. Liquid cultures and colonies are cream to pale yellow. Grow well in ordinary nutrient media containing peptone or in mineral media supplemented with simple organic compounds as electron donors and carbon sources. **Do not utilize sugars as carbon or energy sources.** Mesophilic, neutrophilic, and nonhalophilic. Catalase and oxidase positive. No hydrolysis of polysaccharides, protein, or lipids.

Straight-chain hexadecanoic acids ($C_{16:0}$ and $C_{16:1}$) are the major components of cellular fatty acids. 3-Hydroxydecanoic acid ($C_{10:0\ 3OH}$) is present. Ubiquinones and rhodoquinones with eight isoprene units (Q-8 and RQ-8) are the major respiratory quinones. Isolated from activated sludge systems, such as soybean-curd wastewater sludge and municipal sewage sludge.

The mol% G + C of the DNA is: 63–65.

Type species: **Brachymonas denitrificans** Hiraishi, Shin and Sugiyama 1995b, 879 (Effective publication: Hiraishi, Shin and Sugiyama 1995c, 110.)

FURTHER DESCRIPTIVE INFORMATION

The description of the genus *Brachymonas* is based on only one species, *B. denitrificans*. Morphological features are as shown in Fig. BXII.β.28. Electron microscopy of negatively stained cells shows that the cells have no flagella. Colonies on agar media are of intermediate size (3–5 mm after 3 d), smooth, circular, convex, and cream to pale yellow. Some strains produce mucoid colonies upon subculture, and it is hard to harvest cells from such colonies by ordinary centrifugation. In liquid cultures with shaking, growth occurs as a uniform cell suspension. No flocculent growth is found at any growth stage.
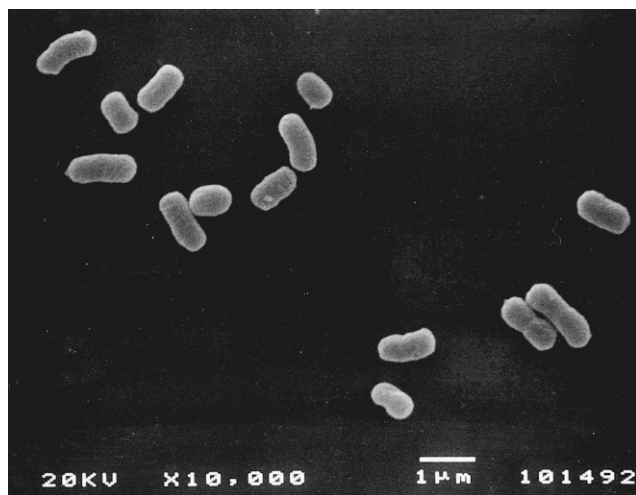

FIGURE BXII.β.28. Scanning electron micrograph showing general cell morphology of *Brachymonas denitrificans* (strain AS-P1[T]).

Cells exhibit a doubling time of 1.5–2.0 h under optimal aerobic growth conditions (in peptone–yeast extract medium or lactate–mineral salt medium). Anaerobic growth with nitrate is somewhat slower (doubling time 2–3 h) and is accompanied with nitrogen gas production. Some strains are also capable of growing anaerobically with trimethylamine-*N*-oxide (TMAO) as the terminal electron acceptor, but growth is much slower (doubling time 9–10 h). No strains grow anaerobically with fumarate as the terminal oxidant, but cell-free extracts from these strains exhibit fumarate-reducing activity with reduced FMN as an electron donor. It is unknown whether this activity is related to the occurrence of the low-potential quinone rhodoquinone or if it has some biological significance. A wide variety of simple organic compounds are used as electron donors and carbon sources for both aerobic and denitrifying growth. Good growth occurs with the salts of organic acids (e.g., acetate, lactate, pyruvate, fumarate, and succinate) and amino acids (e.g., alanine, glutamate, leucine, phenylalanine, and proline). Carbohydrates are never utilized.

The optimal temperature for growth is 30–35°C and the optimal pH is 7.0–7.5. NaCl is not required for optimal growth, but multiplication occurs at NaCl concentrations of up to 3%. Growth factors are not required. Ammonium salts are used as nitrogen sources. Sulfate is assimilated as the sulfur source. The following characteristics are negative: indole production; Voges–Proskauer reaction; urease; phenylalanine deaminase; decarboxylation of arginine, lysine, and ornithine; and hydrolysis of aliginic acid, chitin, starch, gelatin, casein, tributyrin, and Tween 80.

Palmitoleic acid ($C_{16:1}$) and palmitic acid ($C_{16:0}$) are the major components of whole-cell fatty acids, constituting 33–43% and 21–28% of the total content, respectively. In addition, a considerable amount (10–17%) of $C_{18:1}$ acid is present. 3-Hydroxydecanoic acid ($C_{10:0\ 3OH}$) is the main fatty acid component of the outer membranes. The major respiratory quinones are Q-8 and RQ-8. The RQ/Q molar ratio is higher in cells growing in complex media (0.09–0.10) than in those in a chemically defined medium with lactate as the sole carbon source (<0.05). In addition, the RQ/Q ratio is higher in aerobically grown cells than in anaerobically grown cells using nitrate or TMAO as the terminal electron acceptor.

Strains of *B. denitrificans* have hitherto been isolated from activated sludge systems, such as soybean-curd wastewater sludge and municipal sewage sludge (Hiraishi and Komagata, 1989b). In addition, rhodoquinone-producing denitrifying strains that are phenotypically similar to *Brachymonas* have been isolated from photosynthetic wastewater treatment sludge (Hiraishi et al., 1991b). As suggested by their capacity for denitrification, *Brachymonas* strains may play important roles in nitrogen removal and organic matter removal in biological wastewater treatment systems.

ENRICHMENT AND ISOLATION PROCEDURES

Selective isolation and enrichment of *Brachymonas* from the environment are difficult, but the following mineral medium sup-

plemented with 20 mM acetate and 20 mM potassium nitrate is recommended (per liter distilled water): $(NH_4)_2SO_4$, 1 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; trace element solution SL8, 1 ml (Biebl and Pfennig, 1978) (pH 6.8). The medium is incubated anaerobically (under denitrifying conditions).

Brachymonas strains grow well in ordinary complex nutrient media, such as PBY medium (0.5% peptone, 0.2% beef extract, and 0.1% yeast extract) (Hiraishi and Komagata, 1989a). Good growth also occurs in the mineral medium described previously with 20 mM lactate or acetate as the sole carbon source.

MAINTENANCE PROCEDURES

Cultures are well preserved in liquid nitrogen or by lyophilization. Preservation can also be done in a mechanical freezer at $-80°C$.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

The presence of both respiratory quinones Q-8 and RQ-8 is a characteristic feature of *Brachymonas* and can be determined by TLC and reverse-phase HPLC (Hiraishi and Hoshino, 1984). In silica-gel TLC, rhodoquinones migrate just behind ubiquinones and are detectable as a pink to purple band under visible light. The type of rhodoquinone homologs, as well as ubiquinones, can be determined by reverse-phase HPLC with an ODS column and methanol–isopropyl ether (3:1, v/v) as the mobile phase.

DIFFERENTIATION OF THE GENUS *BRACHYMONAS* FROM OTHER GENERA

The genus *Brachymonas* can be differentiated from phenotypically and phylogenetically related genera by the features listed in Table BXII.β.48.

TAXONOMIC COMMENTS

Brachymonas strains were isolated from activated sludge and first designated unidentified rhodoquinone-containing chemoorganotrophic bacteria (Hiraishi and Komagata, 1989b). This was the first demonstration of the existence of rhodoquinone-containing chemoorganotrophic bacteria, as, before this study, rhodoquinones had been known to be distributed only in phototrophic purple bacteria among the procaryotes (Hiraishi and Hoshino, 1984; Hiraishi, 1988b). A phylogenetic analysis based on 16S rDNA sequences has shown that *B. denitrificans* strains form a major cluster in the *Betaproteobacteria*, together with members of the genera *Comamonas*, *Variovorax*, and *Rhodoferax* (Hiraishi et al., 1995b). The nearest phylogenetic neighbor is *Comamonas testosteroni* (95% similarity). Also genomic DNA–DNA hybridization studies have shown that *B. denitrificans* forms a genetically coherent group, in that the levels of hybridization among strains of the species are 74–100%. Based on this phylogenetic evidence, together with the chemotaxonomic information, creation of a new genus and species with the name *Brachymonas denitrificans* was proposed for these strains (Hiraishi et al., 1995b). *Brachymonas* may belong to the family *Comamonadaceae*.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *BRACHYMONAS*

The genus *Brachymonas* includes only one species, *B. denitrificans*, at this time. The general characteristics of *B. denitrificans* are indicated in Table BXII.β.49.

List of species of the genus Brachymonas

1. **Brachymonas denitrificans** Hiraishi, Shin and Sugiyama 1995b, 879[VP] (Effective publication: Hiraishi, Shin and Sugiyama 1995c, 111.)

   de.ni.tri′ fi.cans. M.L. adj. *denitrificans* denitrifying.

   The characteristics are as described for the genus and

indicated in Tables BXII.β.48 and BXII.β.49. The habitat is activated sludge.

   The mol% G + C of the DNA is: 63–65 (HPLC).
   Type strain: AS-P1, JCM 9216.
   GenBank accession number (16S rRNA): D14320.

TABLE BXII.β.48. Differential characteristics of *Brachymonas* and phenotypically and phylogenetically related genera[a]

Characteristic	*Brachymonas*	*Acidovorax*	*Comamonas*	*Hydrogenophaga*	*Variovorax*
Cell shape	Short rods, coccobacilli	Rods	Rods	Rods	Rods
Motility by flagella	−	+	+	+	+
Yellow pigment	d	−	−	+	−
Denitrification	+	−	D	−	−
Growth on glucose	−	D	−	+	+
Major quinone(s)	Q-8, RQ-8	Q-8	Q-8	Q-8	Q-8
Mol% G + C of DNA	63–65	62–66	60–69	65–69	67–69

[a]For symbols see standard definitions; Q-8, ubiquinone-8; RQ-8, rhodoquinone-8.

TABLE BXII.β.49. Other characteristics of *Brachymonas denitrificans*[a]

Characteristic	Result or reaction
Cell size (μm)	$0.6–1.0 \times 0.8–1.5$
Yellow pigment	d
Temperature range for growth (°C)	10–40
pH Range for growth	5–9
Growth with 3% NaCl	+
Growth with 5% NaCl	−
Anaerobic growth with:	
Fumarate, dimethylsulfoxide, HCO_3^-/pyruvate	−
Nitrate	+
TMAO	d
N_2 gas production from nitrate	+
Growth factor requirement	−
Catalase, oxidase	+
Hydrolysis of starch, alginate, chitin, casein, gelatin, tributyrin, Tween 80	−
Electron donors and carbon sources:	
Ethanol, propylene glycol, acetate, butyrate, lactate, pyruvate, succinate, fumarate, malate, glutarate, alanine, asparagine, glutamate, leucine, phenylalanine, proline	+
Propionate, benzoate	d
L-Arabinose, D-xylose, D-ribose, L-rhamnose, D-glucose, D-mannose, D-fructose, cellobiose, lactose, sucrose, adonitol, mannitol, sorbitol, glycerol, methanol, formate, caproate, caprylate, pelargonate, citrate, malonate, tartrate, glycolate, gluconate, aminobutyrate, arginine, glycine, histidine, lysine, ornithine, tryptophan	−
Major fatty acids	$C_{16:0}$, $C_{16:1}$
3-OH fatty acid	$C_{10:0}$
Major quinones	Q-8, RQ-8
Mol% G + C of DNA	63–65

[a]For symbols see standard definitions; Q-8, ubiquinone-8; RQ-8, rhodoquinone-8.

Genus IV. **Delftia** *Wen, Fegan, Hayward, Chakraborty and Sly 1999, 573*[VP]

LINDSAY I. SLY, AIMIN WEN AND MARK FEGAN

Delf'tia. M.L. fem. n. *Delftia* referring to the city of Delft, the site of isolation of the type species, and in recognition of the role of Delft research groups in the development of bacteriology.

Cells are **straight to slightly curved rods**, 0.4–0.8 × 2.5–4.1 μm (occasionally up to 7 μm), occurring **singly or in pairs. Motile by means of polar or bipolar tufts of one to five flagella.** Do not produce endospores. Gram negative. **Oxidase and catalase positive.** No fluorescent pigment produced. Poly-β-hydroxybutyrate is accumulated. **Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Nonfermentative. Reduce nitrate to nitrite. Do not denitrify. **Chemoorganotrophic**. Good growth occurs on media containing organic acids, amino acids, peptone, and carbohydrates. Utilize mannitol and fructose as the sole carbon source, but not glucose. No autotrophic growth with H_2. Hydrolyze acetamide. No levan formation from sucrose. Arginine dihydrolase absent. *Meta* cleavage of protocatechuate. Lipase (Tween 80 hydrolysis) positive. **Main polyamines are putrescine and 2-hydroxyputrescine.** The **major quinone is ubiquinone Q-8**; minor quinones are Q-7 and Q-9. Major fatty acids are hexadecanoic acid ($C_{16:0}$), hexadecenoic acid ($C_{16:1}$), and octadecenoic acid ($C_{18:1}$). 3-hydroxy fatty acids ($C_{10:0\ 3OH}$ and $C_{8:0\ 3OH}$) but not 2-hydroxy fatty acids are present. DNA–rRNA hybridization and 16S rRNA sequence analysis places the genus in the family *Comamonadaceae*. Isolated from soil, sediment, activated sludge, crude oil, oil brine, water, and various clinical samples.

The mol% G + C of the DNA is: 67–69.

Type species: **Delftia acidovorans** (den Dooren de Jong 1926) Wen, Fegan, Hayward, Chakraborty and Sly 1999, 573 (*Pseudomonas acidovorans* den Dooren de Jong 1926, 106; *Comamonas acidovorans* (den Dooren de Jong 1926) Tamaoka, Ha and Komagata 1987, 58.)

FURTHER DESCRIPTIVE INFORMATION

The description of *Delftia* is based on data from a number of studies (Ikemoto et al., 1978b; Palleroni, 1984; De Vos et al., 1985b; Tamaoka et al., 1987; Busse and Auling, 1988; Willems et al., 1989, 1991c, 1992a; Wen et al., 1999).

Growth occurs at 30°C, but not at 4°C and 41°C. Growth occurs in the presence of 0.5 or 1.5% NaCl. No pigments are produced on nutrient agar.

The following organic compounds can be utilized as carbon and energy sources: acetate, acetamide (reported as a variable reaction by Tamaoka et al., 1987 and Willems et al., 1991c), aconitate, adipate, D-alanine (reported as a variable reaction by Willems et al., 1991c), L-alanine (reported as a variable reaction by Willems et al., 1991c), 2-aminobutyrate (reported as a variable reaction by Willems et al., 1991c), δ-aminovalerate, L-aspartate, azelate, butanol, 2,3-butylene glycol, butyrate, caproate (reported as a variable reaction by Willems et al., 1991c), citraconate, citrate (reported as a variable reaction by Willems et al., 1991c), ethanol, D-fructose, fumarate, gluconate, L-glutamate, glutarate, glycerate, glycine (reported as a variable reaction by Willems et al., 1991c), glycolate, hippurate, L-histidine, *m*-hydroxybenzoate, *p*-hydroxybenzoate, β-hydroxybutyrate, hydroxymethylglutarate, isobutyrate, L-isoleucine (reported as a variable reaction by Willems et al., 1991c), isovalerate, itaconate, α-ketoglutarate, kynurenate, L-kynurenine, lactate, levulinate, L-leucine, D-malate (reported as a variable reaction by Willems et al., 1991c), L-malate, maleate (reported as a variable reaction by Willems et al., 1991c), malonate (reported as a variable reaction by Willems et al., 1991c), mannitol, mesaconate, mucate, nicotinate, DL-norleucine (re-

ported as a variable reaction by Willems et al., 1991c), L-norleucine (reported as a variable reaction by Willems et al., 1991c), DL-norvaline, phenylacetate (reported as a variable reaction by Willems et al., 1991c), L-phenylalanine (reported as a variable reaction by Willems et al., 1991c), pimelate, L-proline, n-propanol, propionate, pyruvate, quinate, saccharate, sebacate, suberate, succinate, L(+)-tartrate (reported as a variable reaction by Willems et al., 1991c), m-tartrate, trigonelline (reported as a negative reaction by Willems et al., 1991c), L-tryptophan (reported as a variable reaction by Willems et al., 1991c and Tamaoka et al., 1987), D-tryptophan (reported as a variable reaction by Willems et al., 1991c), L-tyrosine, and valerate.

The following compounds are not utilized: N-acetylglucosamine, adonitol, 3-aminobenzoate, amylamine, anthranilate, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, L-arginine, benzoate (reported as a variable reaction by Willems et al., 1991c), benzoylformate, benzylamine, betaine, butylamine, caprylate, cellobiose, L-citrulline, creatine, L-cysteine, diaminobutane, dodecane, dulcitol, erythritol, esculin, ethanolamine, ethylamine, ethylene glycol (reported as a positive reaction by Tamaoka et al., 1987), D-fucose (reported as a variable reaction by Willems et al., 1991c), D-galactose, β-gentiobiose, geraniol, glucosamine, D-glucose, methyl-α-D-glucoside, glycogen, heptanoate, hexadecane, histamine, o-hydroxybenzoate, poly-β-hydroxybutyrate, DL-β-hydroxybutyrate, inulin, isophthalate, 2-ketogluconate, 5-ketogluconate, lactose, L-lysine, D-lyxose, maltose, D-mandelate, L-mandelate (reported as a variable reaction by Willems et al., 1991c), D-mannose, methyl-α-D-mannoside, D-melezitose, D-melibiose, naphthalene, L-ornithine, oxalate, pantothenate, pelargonate (reported as a variable reaction by Willems et al., 1991c), phenol, phenylethanediol, phthalate (reported as a variable reaction by Willems et al., 1991c), propylene glycol (reported as a positive reaction by Tamaoka et al., 1987), putrescine, D-raffinose, L-rhamnose, D-ribose, salicin, sarcosine (reported as a variable reaction by Willems et al., 1991c), L-serine, sorbitol, L-sorbose, spermine, sucrose, starch, D(−)-tartrate, terephthalate, testosterone, L-threonine (reported as a variable reaction by Willems et al., 1991c and a positive reaction by Tamaoka et al., 1987), trehalose, tryptamine, D-turanose, urea, L-valine (reported as a variable reaction by Willems et al., 1991c), m-xylitol, D-xylose, L-xylose, and methyl-β-D-xyloside.

Variable utilization among different strains occurs with β-alanine, 2-aminobenzoate, 4-aminobenzoate, 3-aminobutyrate, 4-aminobutyrate, 5-aminobutyrate, α-aminovalerate, amygdalin, 2,3-butylene glycol, caprate, m-erythritol, glycerol, heptanoate, m-inositol, isobutanol, DL-kynurenine, L-methionine, and tagatose.

The following characteristics are absent: growth in the presence of 6.5% NaCl, acid production in 10% lactose, in triple sugar iron medium, and in oxidative-fermentative medium containing D-glucose, D-fructose, D-xylose, maltose, or adonitol; production of H_2S in triple sugar iron medium; hydrolysis of esculin,

gelatin, and DNA; indole production, and β-galactosidase activity; hydrolysis of 2-naphthylmyristate, L-valyl-2-naphthylamide, N-benzoyl-DL-arginine-2-naphthylamide, N-glutaryl-phenylalanine-2-naphthylamide, 6-bromo-2-naphthyl-α-D-galactopyranoside, 2-naphthyl-β-D-galactopyranoside, naphthol-AS-BI-β-D-glucuronate, 2-naphthyl-α-D-glucopyranoside, 6-bromo-2-naphthyl-β-D-glucopyranoside, 1-naphthyl-N-acetyl-β-D-glucosaminide, 6-bromo-2-naphthyl-α-D-mannopyranoside, and 2-naphthyl-α-L-fucopyranoside.

ENRICHMENT AND ISOLATION PROCEDURES

The type strain was isolated from soil enriched with acetamide in Delft in the Netherlands in 1926. Since then the bacterium has been shown to be ubiquitous. Strains have been isolated from soil, soil enriched with indole, soil enriched with p-hydroxybenzoate, sediment, activated sludge, sludge enriched with testosterone, crude oil, oil brine, water, and various clinical samples such as urine, pus, and pharyngeal swabs (Willems et al., 1991c). There is increasing evidence of the diverse metabolic capability of *Delftia* in the environment, particularly with respect to the degradation and mineralization of xenobiotic pollutants such as aniline, 2-chloroaniline, and 3-chloroaniline (Ferschl et al., 1991, Hinteregger et al., 1994; Boon et al., 2001), and the enantiomers of 2-(4-sulfophenyl)butyrate (Schulz et al., 2000).

MAINTENANCE PROCEDURES

Cultures are easily maintained on nutrient agar in the laboratory. Cryogenic storage in liquid nitrogen in nutrient broth containing 10% glycerol, and by freeze drying in glucose peptone broth containing horse serum are successful for long-term preservation of *Delftia* cultures.

DIFFERENTIATION OF THE GENUS *DELFTIA* FROM OTHER GENERA

The characteristics for differentiating *Delftia* from other members of the *Comamonadaceae* are given in Table BXII.β.50.

TAXONOMIC COMMENTS

Phylogenetically, *Delftia* belongs to the family *Comamonadaceae* in the class *Betaproteobacteria* according to DNA–rRNA hybridization (Willems et al., 1991a) and rDNA sequence analysis (Wen et al., 1999) (Fig. BXII.β.29). The type species *Delftia acidovorans* belongs to a deep distinct branch. Although the position of the *Delftia acidovorans* branch is not well supported by bootstrap analysis, *D. acidovorans* is consistently the only member of the branch that does not cluster with species of *Comamonas*—where it had been assigned until its transfer to *Delftia* by Wen et al. (1999). The separation of *D. acidovorans* from *Comamonas* is supported by results from DNA–rRNA hybridization (Willems et al., 1991c), 16S rRNA cataloging (Woese et al., 1984a, c), 16S rDNA sequences (Wen et al., 1999), and conventional and chemotaxonomic methods (Tamaoka et al., 1987).

List of species of the genus Delftia

1. **Delftia acidovorans** (den Dooren de Jong 1926) Wen, Fegan, Hayward, Chakraborty and Sly 1999, 573[VP] (*Pseudomonas acidovorans* den Dooren de Jong 1926, 106; *Comamonas acidovorans* (den Dooren de Jong 1926) Tamaoka, Ha and Komagata 1987, 58.)

a.ci.do′vo.rans. L. neut. n. *acidum* acid; L. v. *voro* to devour; M.L. part. adj. *acidovorans* acid devouring.

The characteristics are the same as for the genus and as given in Table BXII.β.50. Strains have been isolated from

soil, sediment, activated sludge, crude oil, oil brine, water, and various clinical samples.

The mol% G + C of the DNA is: 67 (T_m).

Type strain: Stanier 14, ACM 489, ATCC 15668, DSM 39, IAM 12409, IMET 10620, JCM 5833, KS 0057, LMG 1226, NCIB 9681.

GenBank accession number (16S rRNA): AF078774, AB021417.

TABLE BXII.β.50. Differential characteristics of the genus *Delftia* and other genera in the family *Comamonadaceae*[a]

Characteristic	*Delftia*	*Comamonas*[b]	*Acidovorax*[c]	*Hydrogenophaga*[d]	*Variovorax*[e]	*Xylophilus*[f]	*Rhodoferax*[g]	*Brachymonas*[h]	*Polaromonas*[i]	*Aquaspirillum*[j]
Cell shape	Rods	Rods or spirilla	Rods	Rods	Rods	Rods	Curved rods	Cocco-bacilli or short rods	Rods	Spirilla or curved rods
Flagella	Polar or bipolar tufts	Polar or bipolar tufts	1–2 polar	1 polar	Peritrichous	1 polar	1 polar	−	1 polar	Bipolar tufts or 1–2 flagella at only 1 pole
Pigments	−	−	−	+	+	+	−	(+)	−	−
Occurrence:										
Soil	+	+	+	+	+	−	−	−	−	−
Fresh water	+	+	+	+	+	−	−	−	−	+
Seawater	−	−	−	−	−	−	−	−	+	−
Infected plants	−	−	+	−	−	+	−	−	−	−
Clinical samples	+	+	+	−	−	−	−	−	−	−
Activated sludge	+	+	+	−			+	+	−	−
Phototrophy	−	−	−	−			+	−	−	−
Oxidase	+	+	+	+	+	−	+	+	+	+
Chemolithotrophic growth with H_2	−	−	D	+	D	−			−	
Psychrophilic growth	−	−	−	−	−	−	−	−	+	−
Growth factors	−	D	−	−	−	L-Glutamate	Biotin and thiamine	−	−	D
Denitrification	−	−	D	D	−	−	−	+		D
Carbon sources:										
Acetamide	d	−	D	−	−					
β-Alanine	d	−	D	−	d		−			
2-Aminobutyrate	d	−	D	D			−			
3-Aminobutyrate	d	D	D	D			−			
D-Fructose	+	−	+	D		−	+	−		−
D-Glucose	−	−	D	+	+	+	+	−	+	D
Glycerol	d	D	+	+	+	+	−	−	+	D
Malonate	d	−	D	−	d	−		−	−	D
D-Mannitol	+	−	D	D		−	+	−		
Maleate	d	−	D	−	d	−				
Phenylacetate	d	−	−	−	d					
L-(+)-Tartrate	d	−	−	D	d			−		D
D-Tryptophan	d	D	−	−						−
L-Tryptophan	d	−	D	D	d			−		−
Major quinone system:										
Q-8	+	+	+	+	+	+				+
Q-8 + RQ-8							+	+		
Major cellular fatty acid(s):										
$C_{16:0}$	+	+	+	+	+		+	+	+	+
$C_{16:1}$	+	+	+	+	+		+	+		+
$C_{16:1\ \omega7c}$									+	
$C_{18:1}$	+	+	+		+					
$C_{18:1\ \omega7c}$									+	
$C_{18:1\ \omega9t}$									+	
$C_{18:1\ \omega12t}$									+	
Major 3-OH acids:										
$C_{8:0}$	+		+	+			+			
$C_{10:0}$	+	+	+	D	+			+		+
Mol% G + C of DNA	67–69	63–66	67–70	65–69	66–68	68–69	59–61 (HPLC)	63–65 (HPLC)	52–57	56–62

[a]Symbols: +, present in all species; −, absent in all species; (+), weak reaction; d, 11–89% of strains are positive; D, variable reaction in different species.

[b]Data from Palleroni (1984), De Vos et al. (1985b), Tamaoka et al. (1987), and Willems et al. (1991c).

[c]Data from Palleroni (1984) and Willems et al. (1990, 1992a).

[d]Data from Palleroni (1984) and Willems et al. (1989).

[e]Data from Kersters and De Ley (1984b), Willems et al. (1991a), and Urakami et al. (1995a).

[f]Data from Bradbury (1984) and Willems et al. (1987, 1991a).

[g]Data from Hiraishi et al. (1991a).

[h]Data from Hiraishi et al. (1995b).

[i]Data from Irgens et al. (1996).

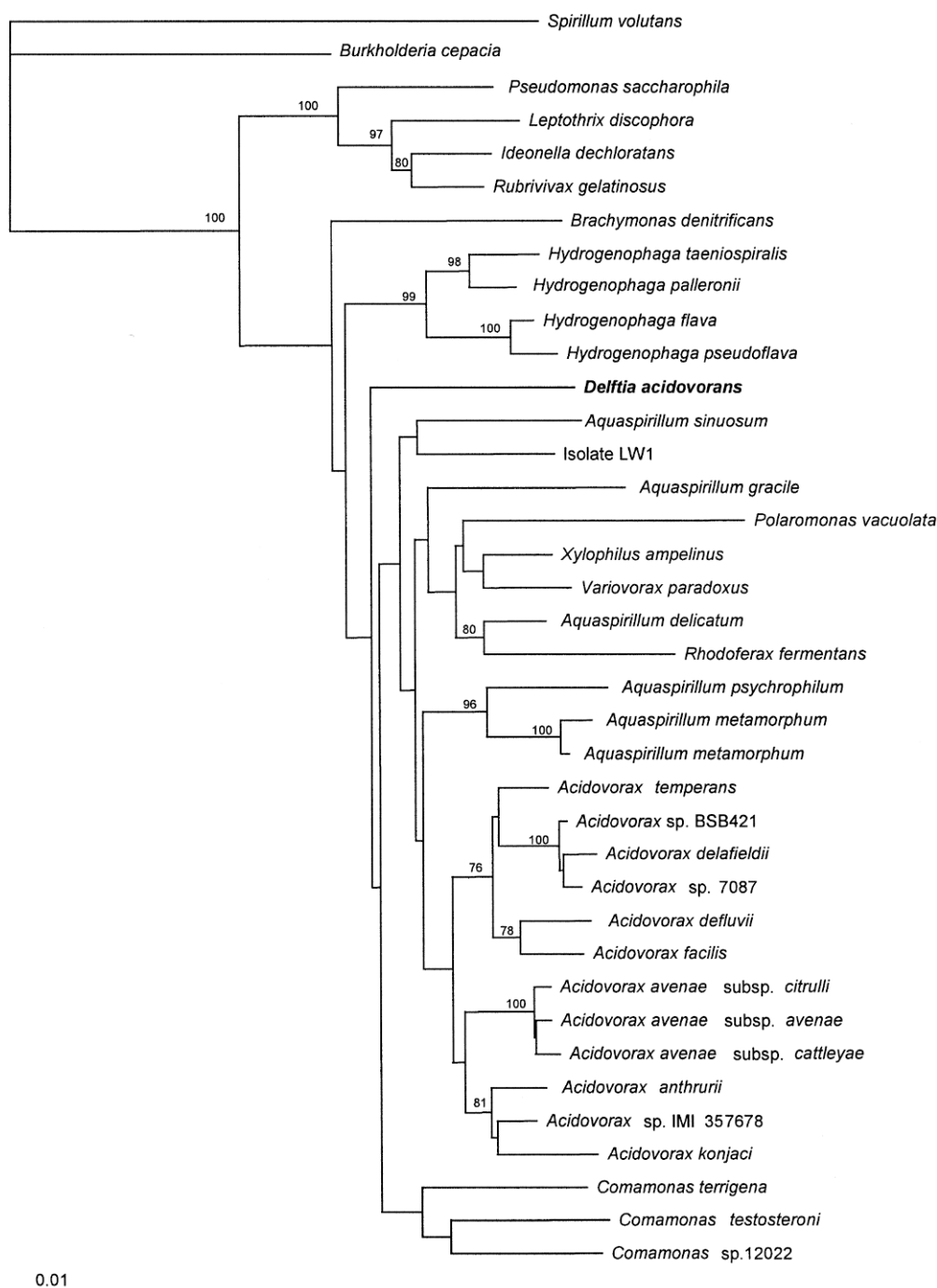[j]Data from Krieg (1984a), Pot et al. (1992a, b), Hamana et al. (1994), and Sakane and Yokota (1994).

FIGURE BXII.β.29. Neighbor-joining tree showing the phylogenetic relationships of *Delftia acidovorans* with members of the family *Comamonadaceae* and related species based on 1290 nucleotide positions of their 16S rDNA sequences. *Burkholderia cepacia* was used as the outgroup. Scale bar represents one nucleotide substitution per 100 nucleotides. Bootstrap values of 100 resamplings are shown at the branch points. Only bootstrap values >75% are shown. The following sequences were used to generate this tree: *Acidovorax avenae* subsp. *avenae* ATCC 19860^T, AF078759; *A. avenae* subsp. *citrulli* ATCC 29625^T, AF078761; *A. avenae* subsp. *cattleyae* NCPPB 961^T, AF078762; *A. konjaci* ATCC 33996^T, AF078760; *A. facilis* CCUG 2113^T, AF078765; *A. temperans* CCUG 11779^T, AF078766; *A. delafieldii* ATCC 17505^T, AF078764; *Acidovorax* sp. IMI 357678, AF078763; *Acidovorax* sp. 7087, AF078767; *Acidovorax anthurii* CFBP 3232^T, AJ007013; *Acidovorax defluvii* BSB411^T, Y18616; *Acidovorax* sp. BSB421, Y18617; *Acidovorax* sp. LW1, AJ130765; *Aquaspirillum gracile* ATCC 19624^T, AF078753; *Aquaspirillum metamorphum* LMG 4339^T, AF078757; *Aquaspirillum metamorphum* DSM 1837^T, Y18618; *Aquaspirillum delicatum* LMG 4328^T, AF078756; *Aquaspirillum psychrophilum* LMG 5408^T, AF078755; *Aquaspirillum sinuosum* LMG 4393^T, AF078754; *Brachymonas denitrificans* JCM 9216^T, D14320; *Burkholderia cepacia* ATCC 25416^T, M22518; *Comamonas testosteroni* ATCC 11996^T, M11224; *Comamonas terrigena* IMI 359870^T, AF078772; *Delftia acidovorans* ACM 489^T, AF078774; *Comamonas* sp. 12022, AF078773; *Hydrogenophaga flava* CCUG 1658^T, AF078771; *H. pseudoflava* ATCC 33668^T, AF078770; *H. palleronii* CCUG 20334^T, AF078769; *H. taeniospiralis* ATCC 49743^T, AF078768; *Ideonella dechloratans* ATCC 15173^T, X72724; *Leptothrix discophora* ATCC 43182, Z18533; *Polaromonas vacuolata* ATCC 51984^T, U14585; *Rhodoferax fermentans* JCM 7819^T, D16211; *Rubrivivax gelatinosus* ATCC 17011^T, D16213; *Spirillum volutans* ATCC 19554, M34131; *Variovorax paradoxus* IAM 12373^T, D30793; *Xylophilus ampelinus* ATCC 33914^T, AF078758; *Pseudomonas saccharophila* ATCC 15946^T, unpublished.

Genus V. **Hydrogenophaga** *Willems, Busse, Goor, Pot, Falsen, Jantzen, Hoste, Gillis, Kersters, Auling and De Ley 1989, 329^{VP}*

ANNE WILLEMS AND MONIQUE GILLIS

Hy.dro.ge.no' pha.ga. Gr. n. *hydro* water; Gr. n. *gennao* to create; M.L. *hydrogenum* hydrogen, that which produces water; Gr. v. *phagein* to eat; M.L. fem. n. *Hydrogenophaga* eater of hydrogen.

Straight to slightly curved rods, 0.3–0.6 × 0.6–5.5 μm, occurring singly or in pairs. **Gram negative. Motile by means of one or, rarely, two polar to subpolar flagella.** Colonies are yellow due to the presence of **carotenoid pigments. Aerobic. Oxidase positive** and **catalase positive**, except for *H. pseudoflava*, which is catalase variable. **Chemoorganotrophic or chemolithoautotrophic**, using the oxidation of H_2 as an energy source and CO_2 as a carbon source. Oxidative carbohydrate metabolism occurs, with oxygen as the terminal electron acceptor; alternatively, two species (*H. pseudoflava* and *H. taeniospiralis*) are capable of **heterotrophic denitrification** of nitrate. Good growth occurs on media containing organic acids, amino acids, or peptone; there is less versatility in the use of carbohydrates. Cellular lipids contain 3-hydroxyoctanoic acid ($C_{8:0\ 3OH}$) either alone or together with 3-hydroxydecanoic acid ($C_{10:0\ 3OH}$); 2-hydroxy-substituted fatty acids are absent. **Ubiquinone Q-8** is the main quinone. Putrescine and 2-hydroxyputrescine are present in approximately equimolar concentrations, either exclusively or as the dominant polyamines.

The mol% G + C of the DNA is: 65–69.

Type species: **Hydrogenophaga flava** (Niklewski 1910) Willems, Busse, Goor, Pot, Falsen, Jantzen, Hoste, Gillis, Kersters, Auling, and De Ley 1989, 329 (*Pseudomonas flava* (Niklewski 1910) Davis, Doudoroff, Stanier, and Mandel 1969, 385; *Hydrogenomonas flava* Niklewski 1910, 123.)

FURTHER DESCRIPTIVE INFORMATION

Flagellation *Hydrogenophaga* cells have one or, rarely, two flagella with polar or subpolar insertion. Aragno et al. (1977) have defined three types of flagella in Gram-negative bacteria based on fine structural details as revealed by electron microscopy. They have reported the flagellar fine structure of *Hydrogenophaga flava* and *Hydrogenophaga palleronii* to be of type I, with flagella having diameters of 13.5–14.0 nm and 14–16 nm, respectively, and wavelengths of 1.2–1.3 μm and 1.2–2.0 μm, respectively. For *Hydrogenophaga pseudoflava*, the flagellar diameter is 13.5–14.0 nm and the wavelength is 1.4–1.9 μm, but the interpretation of flagellar fine structure is uncertain. Pili occur over the total cell surface in *H. flava* and *H. pseudoflava* and are inserted at the polar caps in *H. palleronii* (Aragno et al., 1977) and *Hydrogenophaga taeniospiralis* (Lalucat et al., 1982).

Cell wall composition Walther-Mauruschat et al. (1977) have distinguished three types of cell walls in Gram-negative bacteria, differing mainly in the visibility and location of the peptidoglycan layer. *Hydrogenophaga* possesses type I cell walls, typical of most Gram-negative bacteria, in which the outer membrane and the cytoplasmic membrane are of similar dimensions and appearance and are separated by a dense peptidoglycan layer (Walther-Mauruschat et al., 1977). Cytochromes of the *a*, *b*, and *c* types are present in the membrane fraction, and cytochrome *c* is found in the soluble fraction of autotrophically or heterotrophically grown cells of *H. pseudoflava*, autotrophically grown cells of *H. taeniospiralis*, and heterotrophically grown cells of *H. palleronii* and *H. pseudoflava* (Auling et al., 1978; Lalucat et al., 1982). In addition, *Hydrogenophaga taeniospiralis* contains cytochrome *a* in the soluble fraction (Lalucat et al., 1982), and *H. pseudoflava*

contains small amounts of cytochrome *d* when grown autotrophically (Auling et al., 1978).

Fine structure Intracellular mesosome-like membrane systems with a spiral appearance, often located in the area of cell division or at the cell poles, have been reported in *H. flava*, *H. pseudoflava*, and *H. palleronii* (Walther-Mauruschat et al., 1977). The significance of these structures is unclear, and there are indications they may be artifacts resulting from preparation of cells for electron microscopy. Intracellular inclusions of poly-β-hydroxybutyrate and polyphosphate are present, and translucent glycogen-like inclusions may also be detected (Walther-Mauruschat et al., 1977; Lalucat et al., 1982). *H. taeniospiralis* has been reported to contain R-bodies, which are inclusion bodies consisting of a proteinaceous ribbon that is rolled up to form a hollow cylinder. The function of these structures remains uncertain. Similar structures have been described in some kappa-particles, the endosymbionts in killer paramecia, where they are thought to be involved in toxin release (Lalucat et al., 1982).

Pigments Colonies on nutrient agar are yellow due to the presence of carotenoid pigments. Their absorption maxima in acetone have been reported to be approximately 405, 425, and 446 nm for *H. pseudoflava* (Auling et al., 1978) and *H. palleronii* (Davis et al., 1970; Auling et al., 1978) and approximately 440 and 465 nm for *H. flava* (Davis et al., 1970). More recently, Urakami et al. (1995a) have reported an absorption maximum of 450 nm for the carotenoid pigments of all *Hydrogenophaga* species except *H. taeniospiralis*, for which no absorption spectrum has been determined.

Nutrition and metabolism A variety of organic compounds can be used as sole carbon sources (Tables BXII.β.51 and BXII.β.52). In general, *H. palleronii* is less versatile in its carbohydrate usage than are the other *Hydrogenophaga* species (Willems et al., 1989). The carbohydrate metabolism of *H. pseudoflava* has been studied, and doubling times in various growth conditions have been determined. Glucose and fructose are degraded via the Entner–Doudoroff pathway and 6-phosphogluconate dehydrogenases are not detected in this species (Lee and Schlegel, 1981). Protocatechuate is cleaved via the *meta* mechanism by *H. palleronii* and *H. flava* (Davis et al., 1970); no data are available for the other two species. No organic growth factors are required. All *Hydrogenophaga* species are facultatively chemolithoautotrophic, using the oxidation of hydrogen as an energy source. Hydrogenase is membrane-bound and does not reduce NAD (Aragno and Schlegel, 1992). Some strains of *H. pseudoflava* are capable of nitrogen fixation and carry genes homologous to *Klebsiella pneumoniae nifHDK* genes. It is therefore assumed that the *nif* genes are located on the chromosome (Jenni et al., 1989). Such nitrogen-fixing strains are isolated under heterotrophic selection conditions with combined nitrogen (Jenni et al., 1989). The strains of *H. pseudoflava* that were previously named *"Pseudomonas carboxydoflava"* are capable of autotrophic growth using CO or hydrogen oxidation as an energy source. These organisms can also grow mixotrophically, using the oxidation of CO or hydrogen as an additional energy source while growing hetero-

TABLE BXII.β.51. Characteristics differentiating *Hydrogenophaga* species[a,b]

Characteristic	H. flava	H. palleronii	H. pseudoflava	H. taeniospiralis
Growth on L-arabinose, sucrose, D-galactose, D-fructose, D-mannose, mannitol, D-arabitol, sorbitol, cellobiose, ethanolamine	+	−	+	+
Growth on maltose	+	−	+	−
Growth on L-mandelate, azelate	−	+	+	+
Growth on D-xylose, 4-aminobutyrate, DL-5-aminovalerate, glutarate, L-ornithine, spermine	−	−	+	+
Growth on lactose	−	−	+	−
Growth on 4-hydroxybenzoate	−	+	+	−
Heterotrophic denitrification	−	−	+	+
Reduction of nitrate	+	−	+	+
Reduction of nitrite	−	−	+	+
Hydrolysis of Tween 80	−	+	+	+
Hydrolysis of gelatin	−	−	−	+
Urease	+	−	−	−
Presence of 3-hydroxy-decanoic acid	+	−	+	−

[a]For symbols see standard definitions.

[b]Data from Lalucat et al. (1982), Palleroni (1984), Jenni et al. (1989), and Willems et al. (1989).

trophically (Kiessling and Meyer, 1982). The CO dehydrogenase from *H. pseudoflava* is a molybdenum-containing iron–sulfur flavoprotein with molybdopterin cytosine dinucleotide as the molybdenum cofactor (Tachil and Meyer, 1997). Urothine, which is excreted by *H. pseudoflava*, is produced through the degradation of this cofactor (Volk et al., 1994). *H. pseudoflava* produces poly(3-hydroxybutyrate) when grown on glucose, xylose, or arabinose as the sole carbon source. With propionic acid as a cosubstrate, co-polymers of 3-hydroxybutyric and 3-hydroxyvaleric acids are produced (Bertrand et al., 1990). When grown on combined substrates of glucose and lactones, *H. pseudoflava* strains also accumulate large amounts of copolyesters. The copolyester produced depends on the lactone cosubstrate provided: γ-butyrolactone yields poly(3-hydroxybutyrate-co-4-hydroxybutyrate), while γ-valerolactone yields poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (Choi et al., 1995b). *H. palleronii* strain S1 has been reported to break down 4-aminobenzenesulfonate (sulfanilate) in coculture with *Agrobacterium radiobacter* strain S2. Strain S1 fails to grow as an axenic culture in a mineral medium with the same carbon source, but does grow on this substrate after addition of either a sterile supernatant from strain S2 or a combination of 4-aminobenzoate, biotin, and vitamin B_{12} (Dangmann et al., 1996).

Other biochemical characteristics *Hydrogenophaga* species possess a Q-8 ubiquinone system (Willems et al., 1989; Urakami et al., 1995a). Putrescine and hydroxyputrescine are the main polyamines and are present in approximately equimolar amounts; in addition, *H. pseudoflava* contains small amounts of spermidine and spermine (Willems et al., 1989). Major fatty acids (representing at least 7% of total fatty acids) are palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), and *cis*-vaccenic acid ($C_{18:1\ \omega7c}$). No 2-hydroxy substituted fatty acids are present, but all species contain small amounts (1–5%) of 3-hydroxyoctanoic acid ($C_{8:0\ 3OH}$), and *H. flava* and *H. pseudoflava* contain small amounts (1–6%) of 3-hydroxydecanoic acid ($C_{10:0\ 3OH}$) (Willems et al., 1989; Urakami et al., 1995a). In addition, *H. pseudoflava*, *H. taeniospiralis*, and *H. palleronii* contain about 2%, 5%, and 10–20 %, respectively, of a cyclopropane substituted fatty acid ($C_{17:0\ cyclo}$) (Willems et al., 1989).

Plasmids Two plasmids have been found in "*Pseudomonas carboxydoflava*" (Gerstenberg et al., 1982; Kraut and Meyer, 1988),

now incorporated into *H. pseudoflava*. Plasmids have not been detected in *H. pseudoflava* strains previously classified as *Pseudomonas pseudoflava* (Gerstenberg et al., 1982; Jenni et al., 1989) or in *H. palleronii* (Gerstenberg et al., 1982).

Phages Auling et al. (1978) have tested susceptibility of *H. pseudoflava*, *H. palleronii*, and *H. flava* strains to a number of temperate phages that were isolated originally from autotrophically grown *Pseudomonas pseudoflava* strains. The bacteriophages have a very restricted host range, showing no effects on *H. flava*, *H. palleronii*, *Variovorax paradoxus*, or *Ralstonia eutropha*. Lalucat et al. (1982) have reported that old heterotrophically-grown cultures of *H. taeniospiralis*, when treated with mitomycin C, occasionally contain phage tails and empty phage-head-like particles, and even complete phages with contracted tails in one instance. Suspensions of these structures do not have a lytic effect on any of the four *Hydrogenophaga* species, nor on *V. paradoxus*. The use of ultraviolet light or a temperature shift does not induce the formation of similar phage-like structures. The presence of defective phages in *H. taeniospiralis* may be seen as an argument in favor of the hypothesis that the characteristic R-bodies present in this species may be coded by defective phages, in analogy to a hypothesis put forward to explain the presence of R-bodies in *Caedibacter*, obligate endosymbionts of certain species of paramecia (Lalucat et al., 1982).

Antibiotic sensitivity Most *Hydrogenophaga* species are susceptible to a wide range of antibiotics and bacteriostatic agents (Auling et al., 1978). Table BXII.β.52 contains a detailed listing of antibiotic sensitivities for all species, except *Hydrogenophaga taeniospiralis*, for which few such data are available.

Ecology *Hydrogenophaga* species occur in water, mud, and soil, from which they are usually isolated using enrichment procedures for autotrophic hydrogen oxidizers. All strains isolated are initially capable of autotrophic growth, but some species, such as the type species *Hydrogenophaga flava*, may lose this ability. Nitrogen fixation has been reported in some strains of *H. pseudoflava*, but not in the type strain. *Hydrogenophaga* strains are among the bacteria detected in biofilms in a sand filter used for denitrification of treated waste water and occur in both aerobic and anoxic conditions (Lemmer et al., 1997). *Hydrogenophaga* strains are present in the culturable microbial flora isolated from a waste-oil contaminated site during the course of a 2-year bio-

TABLE BXII.β.52. Additional characteristics of *Hydrogenophaga* species[a, b]

Characteristic	*H. flava*	*H. palleronii*	*H. pseudoflava*	*H. taeniospiralis*
Tolerance to 20% oxygen	−	+	+	nd
Aerobic autotrophic growth with CO	nd	nd	d	−
Growth at:				
37°C	nd	nd	+	+[c]
40°C	nd	nd	+	+
41°C	−	−	+	nd
42°C	nd	nd	−	−
Use of ammonia as sole nitrogen source	+	+	+	nd
Nitrogen fixation	−	−	d	−
Hydrolysis of acetamide, casein, DNA	nd	nd	−	−
Hydrolysis of esculin	+	nd	−[d]	−[d]
Lysine and ornithine decarboxylase	nd	nd	−	−
Production of pyoverdins or phenazine pigments	−	−	−	nd
β-Galactosidase	nd	nd	+	+
Hemolysis	nd	nd	−	−
Growth on:				
Trehalose	+	−	+	+[c]
D-Turanose	+	−	+[c]	−
L-Rhamnose	+	−	v	+
L-Fucose	+	−	−[e]	+
D-Ribose, D-fucose	−	−	−[e]	−
D-Arabinose	−[d,f]	−	−[e]	−
D-Lyxose	nd	nd	d	−
D-Melibiose, D-raffinose, D-tagatose, L-sorbose, L-xylose, melezitose, β-gentiobiose, dulcitol, L-arabitol, xylitol, glycogen, α-methyl-D-glucoside, α-methyl-D-mannoside, α-methylxyloside	nd	nd	−	−
Salicin	−[d,f]	−	+	−
Amygdalin, arbutin, esculin	nd	nd	−[e]	−
Ethanol	+	+	v[f]	nd
n-Propanol	+[g]	+	v[e]	nd
n-Butanol	−	+	v[e]	nd
Isobutanol	−	+	nd	nd
Methanol, phenylethanediol	−	−	nd	nd
Ethylene glycol, 2,3-butylene glycol	−	−	−	nd
Propylene glycol	+	+	nd	nd
Phenol	−	+	+	nd
Testosterone	−	−	−	nd
Acetate	+	+[c,g]	+[e]	−
Propionate	−	−[e,h]	−[f]	−
Butyrate	−[f]	+	v[d,f]	−[d,f]
Isobutyrate	−	−[f]	−	−
Formate	−	−[f]	−	+
Valerate	−	−[e,h]	v[f]	−
Isovalerate	−	−[d,f]	v[c]	−
2-Ketoglutarate	+[c,g]	+[c,g]	v[e]	+
DL-Glycerate	+	+	v[d,f]	+
D(−)-Tartrate	+	+	v[c]	−
L(+)-Tartrate	+[c,g]	+[c,g]	v	−
meso-Tartrate	−	+	−[e]	−
3-Hydroxybutyrate	+	+	+	nd
Glycerate	+	+	nd	nd
Glycolate	−[d]	+	v[f]	−
Citrate	−	+[c,g]	+[e]	−
Aconitate	−	+[c,g]	+[c,g]	−
Levulinate	−[d,f]	−	+	+
m-Hydroxybenzoate	+[g]	+	d	−
Suberate	−	+	v[d,f]	+
Saccharate	−	+	v	nd
Adipate	−	d	+[c]	−
Caproate, oxalate, phthalate, 2-ketogluconate, citraconate, benzoate	−	−	−[e]	−
5-Ketogluconate	nd	nd	−[e]	−
Quinate	−	−	+	nd
Kynurenate, mucate	−	+	nd	nd
Benzoylformate	−	d	nd	nd
Terephthalate	−	−[h]	−	−
Itaconate	−	d	−	−
Pimelate	−	−[e,h]	v[c,f]	−
Sebacate	−	−[d,f]	+	+
Anthranilate, hippurate, hydroxymethylglutarate, nicotinate, pantothenate, poly-β-hydroxybutyrate	−	−	−	nd

(continued)

TABLE BXII.β.52. *(cont.)*

Characteristic	*H. flava*	*H. palleronii*	*H. pseudoflava*	*H. taeniospiralis*
Eicosanedioate	−	−	nd	nd
L-Phenylalanine	+	+[e,h]	+	+
L-Tyrosine	+[e]	+[e,h]	+	+
L-Alanine	+[c,g]	+[e,h]	+[e]	−
D-Alanine	−	+[e,h]	+	−
L-Leucine, L-tryptophan	−	+[e,h]	+	+
L-Isoleucine	−[d,f]	d	+	+
L-Histidine	−[d,f]	−	+	−
L-Threonine, citrulline	−	−	−[e]	−
L-Lysine	−	−	+	+[c,g]
L-Aspargine	−	+	+	nd
Glycine	−	−	v[c,g]	−
L-Arginine	−	−	v	+
L-Valine	−	−	v	−
β-Alanine	−	−	d[c]	−
DL-2-Aminobutyrate	−	−	d	−
DL-3-Aminobutyrate	−	−	−[e]	+
L-Serine, benzylamine	−	−	d	−
DL-Arginine, DL-citrulline, DL-ornithine, methylamine, DL-2-aminovalerate	−	−	nd	nd
L-Cysteine, L-methionine, DL-kynurenine, ethylamine, glucosamine, trigonelin, urea	nd	nd	−	−
L-Norleucine	nd	d	d	−
DL-Norvaline	nd	nd	d	−
Butylamine	+	−	d[d]	+
L-Kynurenine	−	−	−	nd
3-Aminobenzoate, 4-aminobenzoate	−	−	−	−
2-Aminobenzoate, phenylacetate	nd	nd	−	−
N-Acetyl glucosamine	nd	nd	−[e]	−
Amylamine	nd	nd	+	−
Diaminobutane	nd	nd	+	+
Cetrimide	nd	nd	−	−
Growth in the presence of:				
NaCl, 0.5%	nd	nd	+	−
NaCl (1.5%, 3%, 4.5%, 6.5%)	nd	nd	−	−
Aerobic acid production from:				
D-Glucose, maltose	+	−	+[c]	−
Adonitol	nd	nd	−	−
Saccharose, trehalose, cellobiose	+	−	+	nd
D-Fructose	+	−	+[c]	
Arabinose	+	−	+	−
Mannose, galactose, rhamnose	+	−	d	nd
Xylose	−	−	d	−
Fucose	−	−	−	nd
Growth inhibited by:				
SDS, 100 μg/ml	+	+	+	nd
SDS, 50 μg/ml	−	+	−	nd
5% NaCl, 3% Glycine	+	+	+	nd
2% Glycine	−	+	−	nd
3% Tween 20	+	+	+	nd
3% Tween 40, 3% Tween 80	+	+	−	nd
Sodium azide 100 mg/ml	+	+	+	nd
2% Methionine	+	+	−	nd
Susceptibility to:[i]				
Chloramphenicol (30 μg), erythromycin (15 μg), neomycin (30 μg), novobiocin (30 μg), streptomycin (10 μg), tetracycline (30 μg)	+	+	+	nd
Ampicillin (10 μg), kanamycin (30 μg), methicillin (5 μg), polymyxin B (300 U)	+	d	+	nd
Penicillin (10 μg)	nd	nd	+	+

[a]For symbols see standard definitions; nd, not determined.

[b]Data taken from Auling et al. (1978), Aragno and Schlegel (1992), Davis et al. (1969, 1970), Palleroni (1984), and Willems et al. (1989). All strains can grow at 30°C and use nitrate as sole nitrogen source and grow on the following substrates: D-malate, DL-β-hydroxybutyrate, fumarate, pyruvate, succinate, gluconate, lactate, glucose, glycerol, *m*-inositol, L-aspartate, L-glutamate, L-malate, and L-proline. None of the strains hydrolyzes starch or poly-β-hydroxybutyrate, possesses arginine dihydrolase or produces acid from ribose. All strains fail to grow on caprate, caprylate, heptanoate, maleate, malonate, mesaconate, *o*-hydroxybenzoate, pelargonate, D-mandelate, isophthalate, adonitol, erythritol, inulin, starch, D-tryptophan, creatine, betaine, histamine, acetamide, sarcosine, and tryptamine.

[c]Negative according to Willems et al. (1989).

[d]Positive according to Willems et al. (1989).

[e]Mixed reaction reported by Aragno and Schlegel (1992).

[f]Positive according to Aragno and Schlegel (1992).

[g]Negative according to Aragno and Schlegel (1992).

[h]Mixed reaction reported by Willems et al. (1989).

[i] +, inhibition zone >5 mm; −, inhibition zone <2 mm; d, inhibition zone 2–5 mm.

remediation program (Kämpfer et al., 1993). The mineralization and colonization of palmitic acid by *H. pseudoflava* has been studied to investigate the role of bacterial colonization in the degradation of water-insoluble organic compounds (Thomas and Alexander, 1987). In a study on the influence of temperature on the growth rate of and competition among psychrotolerant Antarctic bacteria, *H. pseudoflava* was used as a model organism in a coculture with a *Brevibacterium* strain (Nedwell and Rutter, 1994). Studies of the biphenyl and polychlorinated biphenyl (PCB)-degrading bacteria isolated from a PCB-contaminated site have identified *H. pseudoflava* as a minor component of the microflora dominated by *Comamonas testosteroni* (Joshi and Walia, 1995). A root-growth promoting effect is attributed to *H. pseudoflava* strains occurring in the rhizosphere of hybrid spruce seedlings (Chanway and Holl, 1993).

ENRICHMENT AND ISOLATION PROCEDURES

Facultatively chemolithotrophic H_2-oxidizing strains of *Hydrogenophaga* can be isolated from soil, mud, or water by enrichment in a mineral medium, when incubated under an atmosphere composed of H_2, O_2, and CO_2. Many of the earlier isolates were obtained using the liquid basal mineral medium[1] described by Palleroni and Doudoroff (1972). After inoculation, cultures are incubated at 30°C under an atmosphere of $H_2/O_2/CO_2/N_2$ (50:4–20:5:25–41) (Davis et al., 1970). A varying proportion of *H. pseudoflava* strains isolated under these conditions may be capable of N_2 fixation, despite the presence of organic nitrogen in the medium (Jenni et al., 1989). Nitrogen-fixing *H. pseudoflava* strains have also been isolated from nitrogen-limited soils that were flushed with a gas mixture composed of $H_2/O_2/CO_2/N_2$ (10:5:55:30) (Aragno and Schlegel, 1992). Heterotrophic strains can be isolated from soil by enrichment with pantothenate (Davis et al., 1970) or poly-β-hydroxybutyrate (Delafield et al., 1965). Other strains have been isolated from soil and water samples using various complex chemicals for enrichment (Komagata et al., 1997; Suyama et al., 1998a; Thomas et al., 1998).

MAINTENANCE PROCEDURES

Hydrogenophaga strains can be maintained under autotrophic or heterotrophic conditions, but autotrophic properties may be lost under a prolonged heterotrophic regime, and it may therefore be advisable to maintain cultures under autotrophic conditions. In particular, *H. flava* has been reported to have lost its capacity for autotrophic hydrogen oxidation (Kluyver and Manten, 1942). In general, autotrophic cultures of hydrogen-oxidizing bacteria remain viable for up to 6 months at 4°C, the exception being *H. pseudoflava*, which requires transferring about every 2 months (Aragno and Schlegel, 1992). For heterotrophic growth, convenient media, such as nutrient agar, can be used; for the maintenance of specific metabolic properties, special selective media may be required.

Hydrogenophaga strains can be lyophilized in skim milk in the presence of a suitable cryoprotectant, such as 5% glutamate, 5% *meso*-inositol, or 10% honey (Aragno and Schlegel, 1992). For preservation of the autotrophic properties, autotrophically grown cells should be used for lyophilization. The use of activated charcoal (5%, w/v) in the suspension medium, together with skim milk (20%, w/v) and raffinose or *meso*-inositol (5% w/v),

is also reported to result in good recovery of *Hydrogenophaga* strains after lyophilization (Malik, 1990b).

DIFFERENTIATION OF THE GENUS *HYDROGENOPHAGA* FROM OTHER GENERA

See Table BXII.β.42 for the family *Comamonadaceae* for features differentiating *Hydrogenophaga* from other genera.

An oligonucleotide probe for the genus *Hydrogenophaga*, located in variable region II of the 16S rRNA gene, has been designed and shown to allow differentiation from the species of *Comamonas* and *Acidovorax* when hybridized in the presence of 30% formamide (Amann et al., 1996).

TAXONOMIC COMMENTS

The genus *Hydrogenophaga* consists of four species previously classified in the genus *Pseudomonas*. Earlier still, one of those species, *Pseudomonas flava*, was classified in *Hydrogenomonas*, a large genus containing Gram-negative, facultatively autotrophic, hydrogen bacteria. Davis et al. (1969) proposed that this genus should be abandoned and transferred the various species to other genera. The yellow-pigmented *Hydrogenomonas flava* was transferred to the genus *Pseudomonas*. Several other species of yellow-pigmented hydrogen oxidizers have subsequently been described in the genus *Pseudomonas*. Davis et al. (1970) created *Pseudomonas palleronii*, which differed from *Pseudomonas flava* in its carotenoid pigments and its inability to grow on a number of carbohydrates. Auling et al. (1978) described *Pseudomonas pseudoflava*, a species highly related to *Pseudomonas flava*, and Lalucat et al. (1982) described *Pseudomonas taeniospiralis*, a species containing characteristic R-bodies. By DNA–rRNA hybridizations, the genus *Pseudomonas* was later shown to consist of five distinct groups, which were only remotely related and could not be maintained in a single genus (Palleroni et al., 1973; De Vos and De Ley, 1983). This initiated a gradual removal of species from this genus as new data became available, leading to a classification reflecting phylogenetic relationships. The yellow hydrogen-oxidizing *Pseudomonas* species belonged to the acidovorans rRNA complex, together with a number of other taxa. Based on genotypic, phenotypic, and chemotaxonomic data, it was shown that they were more closely related to each other than to the other members of the acidovorans rRNA complex, and, therefore, a new genus *Hydrogenophaga* was proposed to accommodate them (Willems et al., 1989). More recently, analysis of the 16S rRNA genes of members of the *Comamonadaceae* has confirmed that the genus *Hydrogenophaga* forms a separate cluster in this family (Wen et al., 1999).

Several strains or groups of strains very similar to, but different from, *H. pseudoflava* have been described. They may represent additional separate species, but so far have not been formally named. For instance, Davis et al. (1969) have described *Pseudomonas* strain 450, isolated from soil in Berkeley, California, as being similar to, but phenotypically distinct from, *Pseudomonas flava*. The main differences are its ability to grow autotrophically in the presence of 20% O_2 and its use of a wider variety of carbohydrates for growth. These characteristics also distinguish *H. flava* from *H. pseudoflava* (Auling et al., 1978). A comparison of the metabolic properties of strain 450 and *H. pseudoflava* shows that they are quite similar, the main differences being the absence of denitrification in strain 450 and its inability to use *p*-hydroxybenzoate, phenol, quinate, D-alanine, or L-tryptophan. Auling et al. (1978) did not include this strain in their study when they described *Pseudomonas pseudoflava*, so the precise classification of strain 450 remains uncertain.

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1. Basal mineral medium consists of 0.033 M Na-K phosphate buffer, pH 6.8; 0.1% NH_4Cl; 0.05% $MgSO_4 \cdot 7H_2O$; 0.005% ferric ammonium citrate; and 0.0005% $CaCl_2$ (Palleroni and Doudoroff, 1972).

DNA–DNA hybridizations have confirmed that *H. flava* and *H. pseudoflava* are highly related species, with levels of DNA-binding of 48–62%, whereas *H. palleronii* and *H. taeniospiralis* are clearly separate species, each showing no significant DNA-binding with the other *Hydrogenophaga* species (Willems et al., 1989). No significant DNA binding has been detected between representative strains of *Hydrogenophaga* species and *Variovorax paradoxus*, another yellow-pigmented, hydrogen-oxidizing genus of the *Comamonadaceae* (Willems et al., 1991a). In line with these data, comparison of the 16S rRNA gene sequences of the type strains of the *Hydrogenophaga* species demonstrates that *H. flava* and *H. pseudoflava* are highly related (sequence similarity 99.1%; Wen et al., 1999).

Several strains similar to *Pseudomonas pseudoflava* have been isolated from water of a freshwater eutrophic lake by membrane filtration and incubation on mineral medium containing NH_4Cl and pyruvate under an atmosphere of H_2 and O_2. Most of these strains are capable of N_2 fixation, and by phenotypic comparison and DNA–DNA hybridization, they are similar to, but distinct from, *Pseudomonas pseudoflava* (Jenni et al., 1989). Comparison of published phenotypic data for these strains (Aragno and Schlegel, 1992) with those for strain 450, *H. flava*, and *H. pseudoflava* shows this group to be distinguishable. More genotypic data are required to assess whether it represents a separate species.

FURTHER READING

Aragno, M. and H.G. Schlegel. 1992. The mesophilic hydrogen-oxidizing (Knallgas) bacteria. *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications., 2nd Ed., Vol. 1, Springer-Verlag, New York. pp. 344–384.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *HYDROGENOPHAGA*

Characteristics useful in differentiating the various species of *Hydrogenophaga* are listed in Table BXII.β.52.

List of species of the genus *Hydrogenophaga*

1. **Hydrogenophaga flava** (Niklewski 1910) Willems, Busse, Goor, Falsen, Jantzen, Hoste, Gillis, Kersters, Auling and De Ley 1989, 329[VP] (*Pseudomonas flava* (Niklewski 1910) Davis, Doudoroff, Stanier and Mandel 1969, 385; *Hydrogenomonas flava* Niklewski 1910, 123.)

fla'va. L. fem. adj. *flava* yellow.

The characteristics are as described for the genus and as listed in Tables BXII.β.51 and BXII.β.52. Optimal temperature, 30°C.

The only available strain was isolated from mud from a ditch.

The mol% G + C of the DNA is: 66.7 (T_m).and 67.3 (Bd).

Type strain: ATCC 33667, CCUG 1658, DSM 619, LMG 2185.

GenBank accession number (16S rRNA): AF078771.

Additional Remarks: This strain has been reported to have lost its ability to grow autotrophically soon after isolation (Kluyver and Manten, 1942) and to grow rather slowly and unreliably (Auling et al., 1978). Therefore, *Hydrogenophaga pseudoflava*, a genotypically and protein electrophoretically very similar species, has been suggested as an alternative reference species for the genus (Willems et al., 1989). However, in later experiments the *H. flava* LMG 2185[T] did grow well autotrophically using H_2 as its energy source (A. Willems, unpublished observation).

2. **Hydrogenophaga intermedia** Contzen, Moore, Blümel, Stolz and Kämpfer 2001, 793[VP] (Effective publication: Contzen, Moore, Blümel, Stolz and Kämpfer 2000, 492.)

in.ter.me' di.a. L. neut. adj. *intermedium* intermediate, referring to the phylogenetic position of this organism within the genus *Hydrogenophaga*.

The characteristics are the same as those of the genus. This species is not included in Tables BXII.β.51 and BXII.β.52 because it was published after the completion of the chapter. The description below is taken from the original description (Contzen et al., 2000).

H. intermedia is not able to grow chemolithoautotrophically with H_2. The overall polar lipid pattern is characterized by nearly equal amounts of phosphatidylglycerol, phosphatidylethanolamine, and diphosphatidylglycerol. The fatty acid pattern is typical of the genus *Hydrogenophaga* and includes 3-hydroxyoctanoic acid ($C_{8:0\ 3OH}$). The presence of $C_{19:0\ cyclo\ \omega 8-9}$ differentiates *H. intermedia* from other *Hydrogenophaga* species.

Colonies are circular, entire, slightly convex, smooth, and pale yellow on YPG agar at 25°C. Growth at 40°C, but not at 10°C. The following compounds can be used as sole carbon sources: gluconate, D-mannitol, adipate, lactate, 3-hydroxybutyrate, 2-oxoglutarate, suberate, L-leucine, phenylalanine, L-proline, 3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate. Hydrolysis of L-alanine-pNA positive. The type strain does not utilize: *N*-acetyl-D-glucosamine, L-arabinose, arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, D-melibiose, L-rhamnose, ribose, sucrose, salicin, trehalose, D-xylose, adonitol, inositol, sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, azelate, citrate, fumarate, glutarate, itaconate, D-malate, mesaconate, pyruvate, L-alanine, β-alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-serine, and L-tryptophan as sole carbon sources. Hydrolysis of the following substrates is negative: pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-β-D-galactopyranoside, pNP-β-D-glucuronide, pNP-phenylphosphonate, pNP-phosphorylcholine, 2-deoxythymidine-5′-pNP-phosphate, glutamate-γ-3-carboxy-pNP-ester, and L-proline-pNP.

Features for the differentiation from other *Hydrogenophaga* species include the use of mannitol (negative in *H. palleroni*) and the inability to use the following substrates: L-arabinose, sucrose, D-galactose, D-fructose, D-mannose, sorbitol, and D-cellobiose (positive for *H. flava, H. pseudoflava* and *H. palleroni*), maltose and L-histidine (positive for *H. flava* and *H. pseudoflava*), azelate (positive for *H. pseudoflava, H. taeniospiralis* and *H. palleroni*) and D-xylose (positive for *H. pseudoflava* and *H. taeniospiralis*).

The mol% G + C of the DNA is: 68.6 ± 0.1 (HPLC).

Type strain: S1, DSM 5680.

GenBank accession number (16S rRNA): AF019037.

3. **Hydrogenophaga palleronii** (Davis, Stanier, Doudoroff and Mandel 1970) Willems, Busse, Goor, Falsen, Jantzen, Hoste, Gillis, Kersters, Auling and De Ley 1989, 330^{VP} (*Pseudomonas palleronii* Davis, Stanier, Doudoroff and Mandel 1970, 11.)

pal.le.ro′ ni.i. M.L. gen. n. *palleronii* of Palleroni, named after N.J. Palleroni, who first isolated this organism.

The characteristics are as described for the genus and as listed in Tables BXII.β.51 and BXII.β.52. In addition, some strains have been reported to grow on DL-norleucine (Willems et al., 1989). Optimal temperature is 30°C.

Isolated from soil and water by enrichment for hydrogen autotrophs in minimal media with an atmosphere of $H_2/O_2/CO_2$.

The mol% G + C of the DNA is: 67.3–68.5 (T_m).

Type strain: Stanier 362, ATCC 17724, DSM 63, LMG 2366.

GenBank accession number (16S rRNA): AF019073.

Additional Remarks: This strain contains two stable colony types, which have identical gel electrophoretic protein profiles.

4. **Hydrogenophaga pseudoflava** (Auling, Reh, Lee and Schlegel 1978) Willems, Busse, Goor, Falsen, Jantzen, Hoste, Gillis, Kersters, Auling and De Ley 1989, 330^{VP} (*Pseudomonas pseudoflava* Auling, Reh, Lee and Schlegel 1978, 93.)

pseu.do.fla′ va. Gr. adj. *pseudes* false; L. fem. adj. *flava* yellow; M.L. fem. adj. *pseudoflava* not the true (*Hydrogenophaga*) *flava*, referring to the close relationship to *Hydrogenophaga flava*.

The characteristics are as described for the genus and as listed in Tables BXII.β.51 and BXII.β.52. In addition, none of the strains tested produce indole, H_2S in triple sugar iron medium, or acid from 10% lactose and triple sugar iron medium. All strains fail to grow on allantoin, DL-threonine, salicylate, 2-methyl-2-propanol, and uric acid, but do grow on L-glutamine and shikimic acid.

Some *H. pseudoflava* strains, but not the type strain, are capable of N_2 fixation (Jenni et al., 1989). The strains previously classified as *"Pseudomonas carboxydoflava"* are capable of mixotrophic growth on organic substrates and CO or H_2 and CO_2. For CO-autotrophic growth, the presence of molybdopterin cytosine dinucleotide is required as a cofactor (Meyer and Schlegel, 1983; Volk et al., 1994).

H. pseudoflava is highly related to *H. flava* (DNA homology values 48–62%; Willems et al., 1989), but can be distinguished by its ability to grow at higher temperatures (up to 41°C) and at oxygen concentrations of 20% and by its ability to use a wider variety of substrates for growth (Auling et al., 1978).

Optimal temperature is 35–38°C.

Isolated from soil, mud, or water by liquid enrichment for hydrogen bacteria in an atmosphere consisting of $O_2/CO_2/H_2$ (10:10:80).

The mol% G + C of the DNA is: 66.2–68.6 (T_m).

Type strain: GA3, ATCC 33668, CCUG 13799, DSM 1034, LMG 5945.

GenBank accession number (16S rRNA): AF078770.

5. **Hydrogenophaga taeniospiralis** (Lalucat, Parés and Schlegel 1982) Willems, Busse, Goor, Falsen, Jantzen, Hoste, Gillis, Kersters, Auling and De Ley 1989, 330^{VP} (*Pseudomonas taeniospiralis* Lalucat, Parés and Schlegel 1982, 337.)

tae.ni.o.spi.ra′ lis. Gr. n. *taenia* ribbon; L. adj. *spiralis* coiled; M.L. adj. *taeniospiralis* ribbon coiled, after *Caedibacter taeniospiralis*, an organism with which it shares characteristics.

The characteristics are as described for the genus and as listed in Tables BXII.β.51 and BXII.β.52. In addition, this species does not produce indole or acetoin, or H_2S from cysteine, and it does not grow on D-sorbose. No strains able to fix N_2 have been reported. A variable portion of cells in stationary growth phase may contain inclusions, referred to as R-bodies, which are coiled proteinaceous ribbons with an average diameter of 0.25 μm and an average height of 0.21 μm. Similar structures have been described in *Caedibacter taeniospiralis*, a bacterial endosymbiont of *Paramecium*. These R-bodies are thought to originate from defective prophages and extrachromosomal DNA elements and to be involved in conferring the killer trait upon the host paramecium (Lalucat et al., 1982). The R-bodies of *Caedibacter* species have been shown to be quite different from those of *H. taeniospiralis* (Kanabrocki et al., 1986), but the fact that a defective prophage has been detected in *H. taeniospiralis* indicates a possible similar origin in this species (Lalucat et al., 1982).

Optimal temperature is 37°C.

Isolated from soil in Spain.

The mol% G + C of the DNA is: 65.0 (T_m).

Type strain: 2K1, ATCC 49743, CCUG 15921, DSM 2082, LMG 7170.

GenBank accession number (16S rRNA): AF078768.

Genus VI. **Lampropedia** *Schroeter 1886, 151*^{VP}

R.G.E. MURRAY

Lam.pro.pe′ di.a. Gr. adj. *lampros* bright, radiant; Gr. n. *pedia* a plain, flat country; M.L. fem. n. *Lampropedia* a shining flat sheet (of cells).

Sheets of rounded, almost cubical cells, arranged in square tablets of 16–64 cells, occasionally separated into pairs or tetrads. **Divide synchronously in a sheet and alternately in two planes.** The cells of a tablet are enclosed within a **complex, structured envelope.** Each cell is enclosed in a **Gram-negative type of cell wall**. Intracellular granules of **poly-β-hydroxybutyrate** are prominent. No flagella occur. **Twitching movements** of small groups of cells occur during active growth. **Obligately aerobic**, having a strictly respiratory type of metabolism with oxygen serving as the terminal electron acceptor. **Growth occurs as a thin, hydrophobic, extending pellicle** on the surface of both liquid and solid media. Nonpigmented. Optimal temperature, 30°C. Optimal pH, 7.0. Oxidase and catalase positive. Chemoorganotrophic. **Energy sources are limited to intermediates of the tricarboxylic acid cycle.** Carbohydrates, alcohols, glucosides, and fatty acids are not utilized. Ammonium salts or certain amino acids can serve as sole nitrogen sources. **Vitamins may be required for growth.** The ecological niche is unknown but observations and isolations indicate an environment rich in organic matter.

The mol% G + C of the DNA is: 61 (Bd).

Type species: **Lampropedia hyalina** (Ehrenberg 1832) Schroeter 1886, 151 (*Gonium hyalinum* Ehrenberg 1832, 63.)

FURTHER DESCRIPTIVE INFORMATION

This is a genus based mainly on morphological characteristics, and it consists of a single species, *L. hyalina.* The organism is cultivable and strains are available for study. Three other species have been named in the distant past (de Toni and Trevisan, 1889), including pigmented species, but they have not been re-isolated in the intervening years and have no validity today.

The characteristic appearance of growth is a hydrophobic pellicle consisting of square tablets of Gram-negative cells, apparently dividing synchronously in two planes (Fig. BXII.β.30), forming continuous but rumpled sheets on the surface of media (Kuhn and Starr, 1965; Puttlitz and Seeley, 1968; Seeley, 1974).

The distinctive morphology (Fig. BXII.β.30) allows recognition in its natural surroundings. In rich organic environments, and especially in laboratory media loaded with sodium acetate, the cells are full of poly-β-hydroxybutyrate (PHB) (see Fig. BXII.β.31) as several small granules or one large granule (Kuhn and Starr, 1965).

L. hyalina exhibits a peculiar form of motility involving sudden, irregular shifts in the position of small groups of cells. Pringsheim (1955) and Puttlitz and Seeley (1968) describe this phenomenon, and it has been compared (Pringsheim, 1966) to movements seen in the *Chroococcales* as an argument for relationship to cyanobacteria. However, such a "twitching" motility is also seen in other nonflagellated bacteria and most of them have fimbriae (Henrichsen, 1972). *Lampropedia* strains have not been shown to have either flagella or fimbriae. Twitching, generally (Henrichsen, 1972) and in this case (Puttlitz and Seeley, 1968), is a function of living, metabolizing cultures.

The cell wall is of the Gram-negative type with an outer membrane and a thin, underlying peptidoglycan layer. It has not been isolated and characterized in chemical terms. All layers of this wall intrude at once to separate the sister cells (Fig. BXII.β.32).

The structure of the cells is not remarkable, but the envelope that encloses each tablet of cells has unusual features (Chapman et al., 1963; Murray, 1963; Pangborn and Starr, 1966). The separate tablets of 16, 32, or 64 cells are surrounded by a hexagonal array of complex spindle-shaped units (spacing is 23–26 nm) on a thin continuous but perforated layer (7.5 nm holes; spacing is 13.5–14.5 nm). This highly structured integument bridges over the spaces between the cells in the tablet (Fig. BXII.β.31). The space between cells, between the walls and the structured envelope, and within the intruding septa (Figs. BXII.β.31 and BXII.β.32) contains fibrous materials and is called the intercalated layer. Two layers form the enveloping structure and each have p6 symmetry: an outer complex of three polypeptides joined
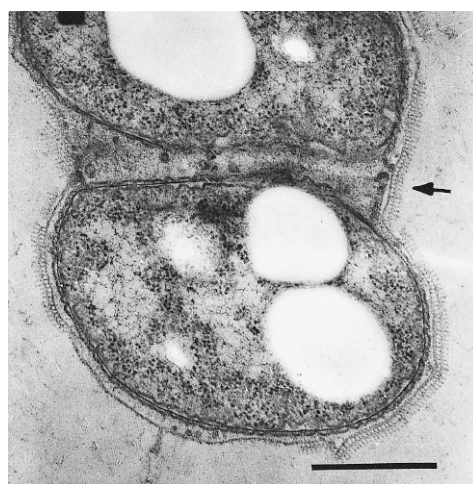

FIGURE BXII.β.31. Section transverse to the edge of a tablet of cells showing a bipartite external structured envelope. The inner layer is obvious in an area exposed by stripping. The envelope encloses the tablet and bridges over the matrix separating adjacent cells (*arrow*). The low density vesicles represent poly-β-hydroxybutyrate granules. Bar = 0.5 μm.
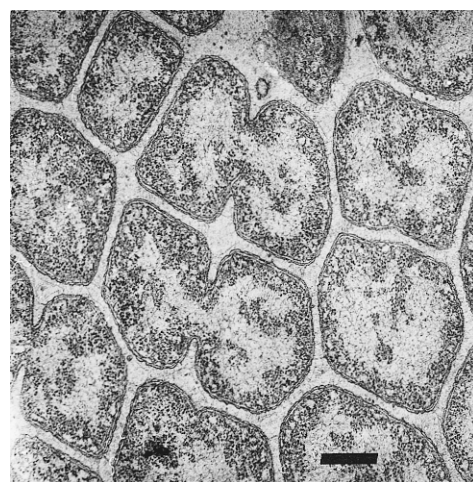

FIGURE BXII.β.30. Light micrograph of a nigrosin preparation of *L. hyalina* showing a corner of a sheet of actively growing tablets of cells. Adjacent tablets are almost synchronized in division. Bar = 5 μm.

FIGURE BXII.β.32. Electron micrograph of a section in the plane of a tablet to show division and the relatively large volume of nucleoplasm. A matrix substance separates the cell walls (of usual Gram-negative character; see Fig. BXII.β.36) of adjacent cells. Divisions always show a "constrictive" form. Bar = 0.5 μm.
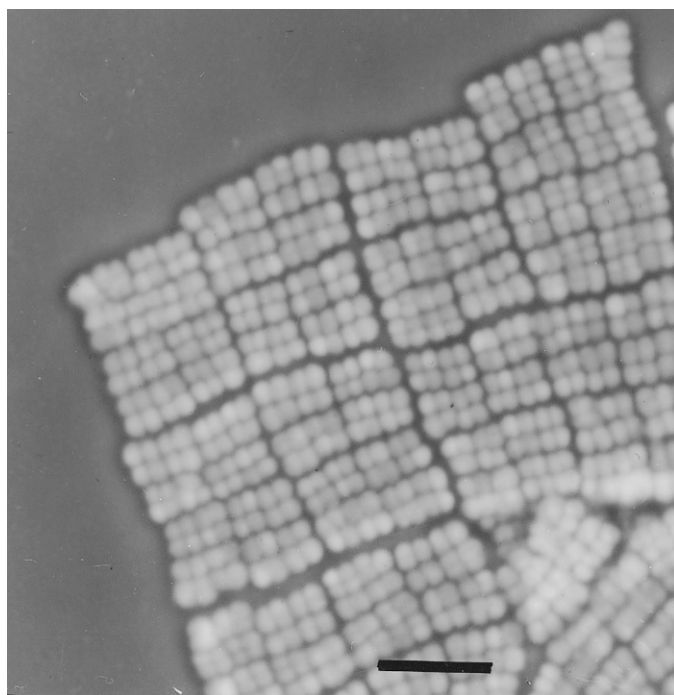
to an inner layer formed by one polypeptide (Austin and Murray, 1987, 1990). The nature of the intercalated materials is unknown. The enveloping layers together accomplish the division and separation of tablets of cells.

Colonial characteristics are determined by the growth habit and the physical nature of the surface of the enveloping layer. The sheets of cells, one cell thick, extend as they grow over the surface of liquid or solid media and tend to wrinkle as they grow to a large size and meet some obstacle to spreading. Many microcolonies are square. An irregularly shaped piece transferred to a liquid medium floats on the surface and maintains that odd shape as it grows; old cultures provide a rain of cells and debris that sink to the bottom. The capacity to float and grow as a pellicle suggests a hydrophobic surface, which is believed to be mediated by fine fibrous material (possibly proteinaceous) external to the envelope array (Lanys, 1972). There is no life cycle.

The description of the genus and its growth characteristics assumes morphological stability. However, mutants are easily derived by isolation of natural variants or stimulated by a mutagen such as nitrosoguanidine (Lanys, 1972, and unpublished results) producing different smooth or rough colonies rather than sheets. Many of these have lost the ability to form tablets or even tetrads, and most have lost the envelope layers (Figs. BXII.β.33 and BXII.β.34). Rough variants usually have retained the envelope arrays (Fig. BXII.β.35), and these most commonly have been derived with the help of nitrosoguanidine. Therefore, it is possible—even probable—that clones exist in nature that are phylogenetically related to *Lampropedia* but show none of the unique morphological characteristics that we use to identify *L. hyalina* in the absence of clear metabolic distinctions. The cultural, physiological, and biochemical characteristics of the available strains have been studied by Puttlitz and Seeley (1968). It is evident that this respiratory, catalase-positive chemoorganotroph requires Krebs cycle intermediates as energy sources and utilizes ammonium salts and a limited palette of amino acids as nitrogen sources. It is slightly fastidious in that it requires biotin and thiamine for growth, but can be cultured in a simple defined me-

dium[1] if these vitamins are supplied. It is a mesophile that grows best at neutral pH and does not tolerate either 0.5% bile or 1.5% salt. It does not produce exoenzymes that degrade proteins, lipids, or the major carbohydrate polymers. So that without its peculiar morphology it is not a distinguished aerobe and would be hard to recognize (see Figs. BXII.β.33, BXII.β.34, and BXII.β.35).

Nothing is known about the genetics, antigenic structure or antibiotic sensitivity of this organism. The mol% G + C of the DNA (M. Mandel, personal communication) ranges from 60.7–61.2 for all available strains.

The ecological niche is unknown. The microbe is sufficiently distinctive (Starr and Skerman, 1965) to be recognized in its tablet or sheeting form by simple microscopy of natural specimens, and undoubtedly it has been seen many times (Starr, 1981), even if there have been no more than three isolations (Pringsheim, 1955; Frank Kovács' strain "Mac 583", described by Schad et al., 1964; Julius Kirchner, noted by Hungate, 1966). The isolations and sightings (sometimes called "window-pane sarcinas") involved waters infused with quantities of organic material, probably well digested and populated with many other microbes. A partial listing includes waters polluted with sugar refinery wastes, swamp water (Schroeter, 1886), stagnant water including aquatic plants (de Toni and Trevisan, 1889), liquid manure from a barnyard (Pringsheim, 1955), rumen fluid (see Smiles and Dobson, 1956; Eadie, 1962; Hungate, 1966; Clarke, 1979), intestinal content of herbivorous reptiles and their nematodes (Schad et al., 1964), and sewage-polluted, muddy water (R. Kolkwitz, 1909, cited by Starr, 1981). Considering the need for organic acids (Krebs cycle intermediates) and for vitamins, as well as the lack of exoenzymes and growth as a pellicle at the air/water interface, it is not surprising that it thrives in many of these situations. Its real habitat remains to be discovered and could be many other places accessible to these sources, perhaps associated with plants or the soil around them. It is hard to believe that this strict aerobe can be even an irregular inhabitant of cattle or sheep rumen unless the lampropedias found in this site can use some hydrogen acceptor other than oxygen (R.E. Hungate, personal communication). However, there is no doubt of the occasional presence of enough window-pane sarcinas to be detected by microscopy.

ENRICHMENT AND ISOLATION PROCEDURES

L. hyalina will grow on many rich media (both liquid and solidified with agar), but isolation is made difficult by enormous numbers of other bacteria in the source material. Pringsheim (1955) used a capillary to transfer individual tablets of cells to dishes with water over a layer of soil in which starch particles or a grain of wheat was embedded; the lampropedias multiplied as a pellicle in the surface film, and, after 1–2 d at room temperature, could be streaked on an agar medium (0.1% sodium acetate, 0.2% yeast extract, and 0.1% peptone) to give a few isolated microcolonies. Microcolonies can be identified readily by low-power microscopy and subcultured. Kirschner (cited by Hungate, 1966) left rumen fluid standing for several days in an open flask and isolated *Lampropedia* by streaking the pellicle that formed onto rumen fluid

FIGURE BXII.β.33. Light micrograph of a nigrosin preparation of a nonsheeting strain derived from the original isolation of *L. hyalina* ATCC strain 11041 (Murray, 1963). Bar = 5.0 μm.

1. The chemically defined medium for growth of *L. hyalina* (Puttlitz and Seeley, 1968) consists of the following (per 1 distilled water): basal salts solution (CaCl$_2$·2H$_2$O, 0.1 g; FeSO$_4$·7H$_2$O, 0.2 g; MgSO$_4$·7H$_2$O, 0.2 g; MnCl$_2$·4H$_2$O, 0.25 g; distilled water, 1000 ml), 100 ml; sodium pyruvate, 3.0 g; NH$_4$Cl, 3.0 g; phosphate buffer solution (KH$_2$PO$_4$, 7.0 g; K$_2$HPO$_4$, 13.0 g; distilled water, 1000 ml), 50 ml; thiamine-HCl, 1.0 mg; biotin, 1.0 mg; and NaOH, 70 mg. The vitamins are added aseptically to the sterile basal medium from stock solutions sterilized by filtration.

FIGURE BXII.β.34. A derivative of *L. hyalina* ATCC 11041 in section showing that it is almost, if not completely, devoid of the enveloping layers. Bar = 0.5 μm.
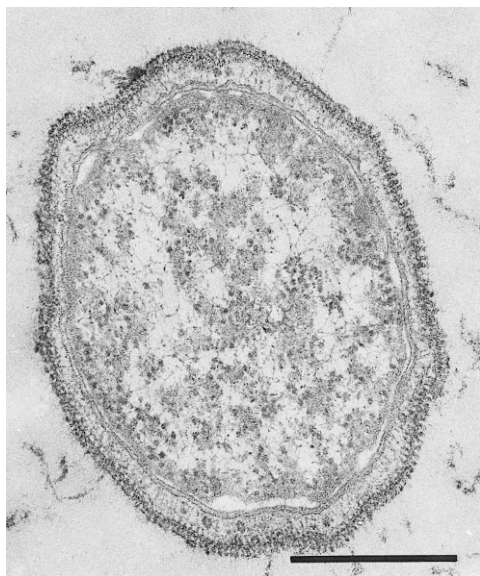

FIGURE BXII.β.35. Electron micrograph of a section of a nonsheeting derivative of *L. hyalina* ATCC 13691, which possesses both the structured and the intercalated enveloping layers outside the cell wall. Bar = 5 μm.

agar, which was then incubated aerobically at room temperature. There are a number of remarks on pellicle formation in the literature, so it seems likely that simple retention at room temperature is the preliminary approach to enrichment.

MAINTENANCE PROCEDURES

The common laboratory practice is maintenance by monthly subculture at room temperature on a neutral pH yeast extract-acetate-peptone agar (0.1–0.5% of each). The organisms survive lyophilization to a fair degree. Undoubtedly, constant subculture

can lead to selection of mutants. Murray (1963) observed that ATCC strain 11041, derived from Pringsheim's isolation, had lost its ability to form sheets (Figs. BXII.β.33 and BXII.β.34). Nowadays, safe stocks can be kept in liquid N_2 suspended in the above fluid medium with 20% glycerol added.

DIFFERENTIATION OF THE GENUS *LAMPROPEDIA* FROM OTHER GENERA

The ability of *Lampropedia* to grow as sheets of cells on liquid or solid media distinguishes it from other genera of nonphotosynthetic bacteria. An organism known as *Thiopedia rosea* has a morphological resemblance to *Lampropedia*, but is photosynthetic (see Taxonomic Comments, below).

TAXONOMIC COMMENTS

Sequence analysis of rDNA shows that *L. hyalina* belongs among the *Betaproteobacteria* (T.M. Schmidt, personal communication) in a grouping that includes *Comamonas*, *Brachymonas*, and more distantly *Leptothrix* and *Variovorax*. However, some aspects of its unique tablet-forming morphology are repeated in *Thiopedia rosea*, a nonsulfur purple, photosynthetic bacterium (Hirsch, 1977b). A similar type of photosynthetic organism was studied in the electron microscope (Lauritis, 1967) and identified as *"Rhodothece"* (now *Amoebobacter*), which did not get beyond the diplococcal form. The extraordinary feature of this organism was the possession of a superficial wall array virtually identical in fine structure to the complex envelope array of *L. hyalina*. In fact, apart from the chromatophore membranes it was similar to some of the enveloped but nonsheeting variants of *L. hyalina* (Fig. BXII.β.35; S.G. Lanys and R.G.E. Murray, unpublished observations). Natural variants isolated from cultures often lose the enveloping structures and the ability to form tablets (Murray, 1963). Associations dependent upon such similarities are fraught with danger because we have no understanding of the genetics of such structures and whether or not the determinants are ge-

netically transferable to any receptive organisms. It is probable that at least one of the old named species, "*L. violacea*" (see Seeley, 1974), may have been one of the *Chromatiaceae* (such as *Lamprocystis roseopersicina*) because the color of cell suspensions is purple to purple-violet (Pfennig and Trüper, 1974).

No less a problem is presented by Pringsheim's (1966) proposal that *L. hyalina* should be regarded as an apochlorotic species of *Merismopedia*, a large coccoid cyanobacterium that forms squared sheets of cells embedded in a glutinous matrix but without evidence of an enveloping structured array. Somewhat similar associations are evident in *Thiocapsa* and *Lamprocystis* among the *Chromatiaceae*.

Few, if any, of these taxonomic hypotheses can be given any credence without strong supportive evidence based, in all probability, on several accepted molecular markers for phylogenetic

relationship. Furthermore, comparative studies of fine structure of a wider range of genera, as well as studies, if possible, of heterotrophically grown *Thiopedia* have yet to be undertaken. Uncertainty about taxonomic associations is exemplified by association with sulfur bacteria in the sixth edition of *Bergey's Manual of Determinative Bacteriology*, complete omission from the seventh edition, and relegation to a genus of uncertain affiliation among Gram-negative cocci in the eighth edition. Obviously, we are no further ahead now, but the tools to solve these problems may be available.

FURTHER READING

Starr, M.P. 1981. The Genus *Lampropedia*. *In* Starr, Stolp, Trüper, Balows and Schlegel (Editors), The Prokaryotes: a Handbook of Habitats, Isolation and Identification of Bacteria, Springer-Verlag, Berlin. pp. 1530–1536.

List of species of the genus Lampropedia

1. **Lampropedia hyalina** (Ehrenberg 1832) Schroeter 1886, 161[AL] (*Gonium hyalinum* Ehrenberg 1832, 63.)
   hya.li′ na. Gr. adj. *hyalinos* glassy, shiny; M.L. fem. adj. *hyalina* hyaline.

   Cells are 1.0–1.5 × 1.0–2.5 μm. Morphological characteristics are as described for the genus and as depicted in Figs. BXII.β.30, BXII.β.31, BXII.β.32, and BXII.β.36.

   Physiological and histochemical characteristics are as described for the genus and as listed in Table BXII.β.53. Utilizes pyruvate, lactate, butyrate, fumarate, malate, succinate

(and acetate in the presence of catalytic levels of pyruvate) as sole energy sources. Utilizes NH_2Cl, alanine, arginine, and tyrosine as sole nitrogen sources. Biotin and thiamine are required for growth.

Temperature range for growth, 10–35°C; optimum, 30°C. pH range for growth, 6.0–8.6, optimum, 7.0.

The mol% G + C of the DNA is: 60.7–61.2 (Bd; M. Mandel, personal communication).

Type strain: ATCC 11041.

Additional Remarks: The type strain is inappropriate because it has lost the characteristic of forming tablets and sheets of cells, and has lost the ability to cover its surface with a structured envelope (Murray, 1963). A possible candidate for a neotype is reference strain ATCC 13871.
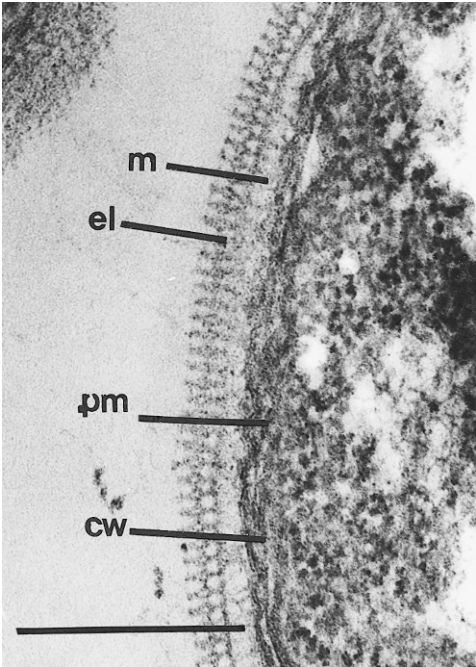

FIGURE BXII.β.36. A high magnification of a section showing the complex structured envelope (*el*), the envelope matrix (*m*), the cell wall (*cw*), and plasma membrane (*pm*). Bar = 0.5 μm.

TABLE BXII.β.53. Physiological and biochemical characteristics of *Lampropedia hyalina*[a,b]

Characteristic	Reaction or result
Growth under anaerobic conditions	−
Intracellular poly-β-hydroxybutyrate formed	+
Oxidase test	+
Catalase test	+
Indole production	−
Acetyl methyl carbinol production (Voges–Proskauer test)	−
Litmus milk	No change
Benzidine test for heme groups	+
Hemolysis on blood agar	−
Arginine deaminase	+
Hydrolysis of gelatin, casein, fats and fatty acids, starch, hippurate, DNA and urea	−
Growth in the presence of:	
1.0% NaCl	+
1.5% NaCl	−
2.0% sucrose	+
4.0% sucrose	−
0.5% bile	−
Final pH in culture media	8.4–8.6

[a]For symbols see standard definitions.

[b]Data from Puttlitz and Seeley (1968).

Other Organisms

It is possible that the "window-pane sarcina" seen in rumen contents (which usually do not show poly-β-hydroxybutyrate granules; R.E. Hungate, personal communication) is separable from *L. hyalina*, but recognition would require cultivation and meta-

bolic studies. An early sighting gave rise to the name *"Bacterium merismopedioides"* (Zopf, 1883), which might be an appropriate name if a rumen strain is ever differentiated from *L. hyalina.*

Many other synonyms probably have been created. Among them the generic name *"Pedioplana"* (Wolff 1907, 10) was considered to be synonymous with *Lampropedia* by Seeley (1974) but, as Starr (1981) points out, it was described as motile and as possessing flagella.

The *species incertae sedis* included by Seeley (1974) are no longer valid, but memory of them should not be erased because they may represent other physiological variations for rediscovery: *"Lampropedia reitenbachii"* (Caspary) de Toni and Trevisan 1889, 1048; *"Lampropedia violacea"* (Brébisson) de Toni and Trevisan 1889, 1048; and *"Lampropedia ochracea"* (Mattenheimer) de Toni and Trevisan 1889, 1049.

Genus VII. Macromonas Utermöhl and Koppe in Koppe 1924, 632[AL]

GALINA A. DUBININA, FRED A. RAINEY AND J. GIJS KUENEN

Ma' cro.mo' nas. Gr. adj. *macrus* large; Gr. n. *monas* a unit, monad; M.L. fem. n. *Macromonas* a large monad.

Large cells are colorless, cylindrical, bean-shaped, or slightly bent. Gram negative. Sluggish or rapidly motile by means of a polar tuft of flagella. Strictly aerobic or microaerophilic. A typical characteristic is the presence of several large inclusions of calcium oxalate, previously believed to be calcium carbonate. Sulfur globules may also be present. Multiplication by constriction followed by fission. No resting stages are known. The genus is composed of two species; the type species has not been grown in pure culture. Several strains of the second species have been studied in pure culture.

Phylogenetic analyses based on 16S DNA sequence comparisons place **Macromonas bipunctata** within the *Betaproteobacteria.* Closest related validly described taxa are the species of the genus *Hydrogenophaga.*

Type species: **Macromonas mobilis** (Lauterborn 1915) Utermöhl and Koppe *in* Koppe 1924, 632 (*"Achromatium mobile"* Lauterborn 1915)

FURTHER DESCRIPTIVE INFORMATION

Cells of the two species are morphologically similar, but differ markedly in size (Table BXII.β.54). *Macromonas bipunctata* cells that lack large inclusions move rapidly. However, as the number of inclusions increases, the motility rate slows down to 600–800 μm/min, which is probably a result of the increased specific gravity of the cells. Between one and four large, or numerous smaller, optically dense inclusions may almost fill the cell (Fig. BXII.β.37). The nature of the refractile intracellular inclusions can be determined by infrared spectroscopy. Absorption spectra of *M. bipunctata* cells grown on medium with succinate have maxima at 1600 and 1300 cm^{-1}, which are typical of the carboxyl groups of oxalate. The polar tuft of flagella ranges in length from 10–40 μm and can sometimes be seen under the light microscope.

Aerobic, aerotactic. Strains of *M. bipunctata* studied thus far are heterotrophs, but can partially oxidize sulfide and thiosulfate by means of hydrogen peroxide.

Both species are found in freshwater environments with low oxygen and hydrogen sulfide concentrations, including hypolimnia, chemoclines, the upper layers of the mud in lakes and ponds, and the surface of sediment in sewage treatment plants. *Macromonas mobilis* has also been reported in acid bog waters (Schultz and Hirsch, 1973).

ENRICHMENT AND ISOLATION PROCEDURES

No methods are known for the isolation and maintenance of cultures of the type species *M. mobilis. M. bipunctata* can be enriched and isolated by the following procedure (Dubinina and Grabovich, 1984). A suitable inoculum (for example, the white mat found on the surface of bottom sediment in a sewage aeration tank) is added to tubes containing 10 ml portions of the following semisolid medium (g/l distilled water): sodium acetate, 1; CaCl$_2$, 0.1; casein hydrolysate, 0.1; yeast extract, 0.1; agar, 1.0. After sterilization, vitamins and trace elements (Pfennig and Lippert, 1966) are added, along with 200 mg freshly precipitated FeS; pH 7.2–7.4. After 2–3 d at 28°C, a white surface film should appear. A suspension of this film is streaked on plates containing the above medium solidified with 10 g agar/l. After 2–3 d, flat colonies of *Macromonas* should appear, and these can be subcultured on solid medium.

MAINTENANCE PROCEDURES

Cultures of *M. bipunctata* can be maintained in semisolid (0.15% agar) medium without FeS but supplemented with thiosulfate (1 g/l), but they should be transferred at about one-month intervals. Lyophilization with cells suspended in skimmed milk has proven to be unsuccessful: the cells remain viable for no more than one or two months.

TAXONOMIC COMMENTS

Phylogenetic analyses based on 16S rDNA–RNA sequence comparisons demonstrate that *Macromonas bipunctata* belongs to the *Betaproteobacteria* and clusters within the radiation of the genera *Hydrogenophaga, Comamonas, Acidovorax, Variovorax,* and *Rhodoferax,* with its closest relatives being the species of the genus *Hydrogenophaga* (Fig. BXII.β.38).

The 16S rDNA sequence of *Macromonas bipunctata* strain VKM 1366^T has 92.8–96.2% similarity to the 16S rDNA sequences of the type strains of each of the species of the genera *Hydrogenophaga, Comamonas, Acidovorax, Variovorax,* and *Rhodoferax.* The highest 16S rDNA sequence similarities of the *Macromonas bipunctata* sequence are found to members of the genus *Hydrogenophaga*: 95.9% to *H. taeniospiralis,* 95.9% to *H. pseudoflava,* 96.1% to *H. flava,* and 96.2% to *H. palleronii.* The 16S rDNA sequence similarity values between the species of the genus *Hydrogenophaga*

TABLE BXII.β.54. Differentiation between *M. mobilis* and *M. bipunctata*

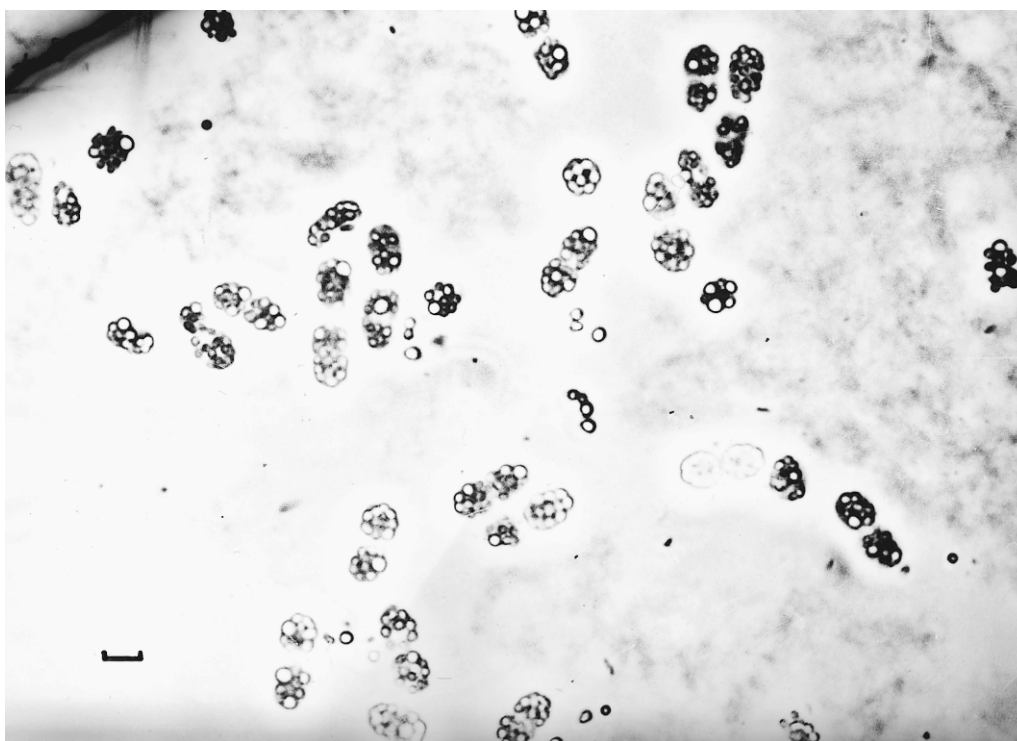| Characteristic	*M. mobilis*	*M. bipunctata*
Cell size	6–14 × 10–30 μm	2.2–4 × 3.3–6.5 μm

FIGURE BXII.β.37. Phase-contrast and electron micrographs of *M. bipunctata* VKM 1366^T showing inclusions of calcium oxalates in cells grown on medium with succinate; Bar = 5 μm.

are in the range of 97.3–99.0%, and so, as shown in the phylogenetic dendrogram (Fig. BXII.β.38), *Macromonas bipunctata* is related to the *Hydrogenophaga* species cluster, but branches off at the deepest point of the cluster.

Although the phylogenetic position of the species *Macromonas bipunctata* is clearly demonstrated by the 16S rDNA analysis of the type strain VKM 1366^T (which exists as a pure culture), the phylogenetic position of *Macromonas mobilis* (the as yet uncultured type species of the genus) is still unknown. Until the 16S rDNA sequence of *Macromonas mobilis* is determined and analyzed, the phylogenetic position of the genus is unclear. However, considering the high degree of similarity between *Macromonas bipunctata* and *Macromonas mobilis* in terms of morphology and ecology, the genus *Macromonas* is probably a member of the *Betaproteobacteria*, with a close phylogenetic relationship to species of the genus *Hydrogenophaga*. Future comparisons of the physiological characteristics of *Macromonas bipunctata* are required to further establish the relationship to the genus *Hydrogenophaga*.

FURTHER READING

la Rivière, J.W.M. and K. Schmidt. 1981. Morphologically conspicuous sulfur-oxidizing bacteria. *In* Starr, Stolp, Trüper, Balows and Schlegel (Editors), The Prokaryotes: a Handbook on Habits, Isolation and Identification of Bacteria, Springer-Verlag, Berlin. pp. 1037–1048.
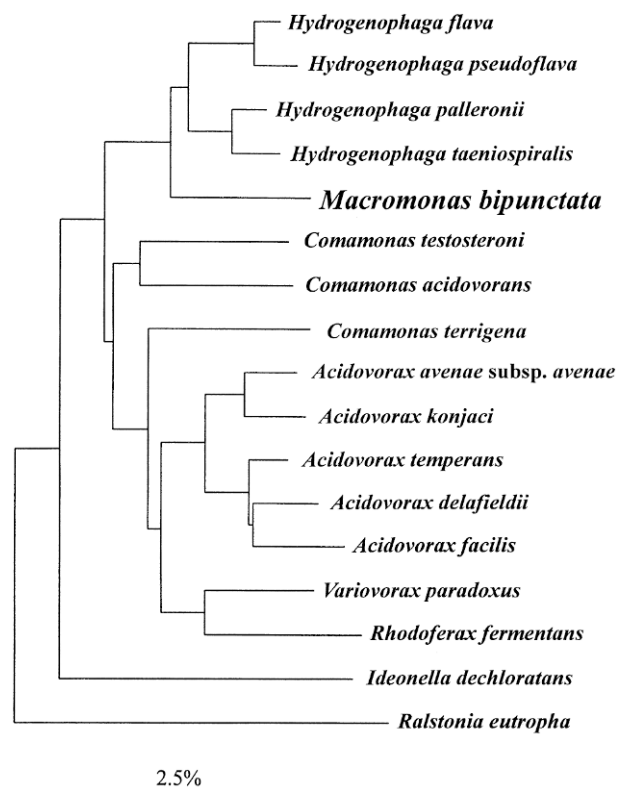

FIGURE BXII.β.38. Phylogenetic dendrogram, based on comparison of 16S rDNA sequences, indicating the position of *Macromonas bipunctata* strain VKM 1366^T and its closest relatives within the *Betaproteobacteria*. The dendrogram was reconstructed from evolutionary distances (Jukes and Cantor, 1969) by the neighbor-joining method (Saitou and Nei, 1987). Bar = 2.5 inferred nucleotide changes per 100 nucleotides.

List of species of the genus Macromonas

1. **Macromonas mobilis** (Lauterborn 1915) Utermöhl and Koppe *in* Koppe 1924, 632[AL] (*"Achromatium mobile"* Lauterborn 1915)

mo' bi.lis L. fem. adj. *mobilis* motile.

The main feature by which this species is currently distinguished from *M. bipunctata* is its large cell size, which is usually 9 × 20 μm, and sometimes 6–14 × 10–30 μm. Its polar tuft of flagella is 20–40 μm long. For further description see that of genus.

The mol% G + C of the DNA is: unavailable.

Type strain: no culture isolated.

2. **Macromonas bipunctata** (ex Utermöhl and Koppe *in* Koppe 1924, 632) Dubinina and Grabovich 1989, 496[VP] (Effective publication: Dubinina and Grabovich 1984, 754.)

bi.punc.ta' ta. L. *bis* twice; L. part. adj. *punctatus* punctate, dotted; M.L. fem. adj. *bipunctata* twice punctate.

Cells single or in pairs, pear-shaped, cylindrical or slightly curved, 2.2–4 × 3.3–6.5 μm, motile by means of a polar tuft of flagella made up of 16–20 fine flagella and ranging in length from 20–30 μm.

Electron micrographs of ultrathin sections of *M. bipunctata* cells reveal the typical structure of Gram-negative bacteria. The cytoplasm is surrounded by a three-layered cell membrane that occasionally forms invaginations. The five-layered cell wall, with a thin layer of peptidoglycan, is separated from the protoplast by a periplasmic space. The cells are covered by a thin layer of fibrillar polysaccharide. In cells grown under intense aeration, this layer may increase in thickness up to 200–500 nm and form slime capsules (Fig. BXII.β.39).

At least four types of inclusion have been observed in *M. bipunctata* cells. These are calcium oxalate, calcium poly-
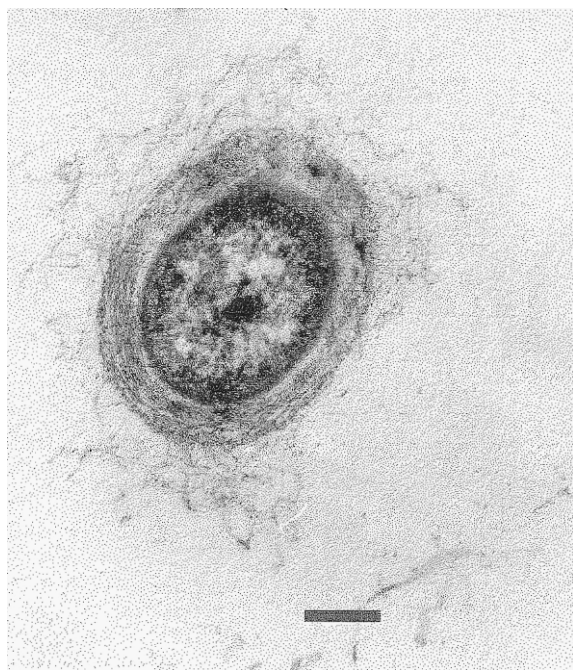

FIGURE BXII.β.39. Electron micrographs of ultrathin sections of ruthenium red-stained *M. bipunctata* VKM 1366[T]. The ruthenium red has stained the extracellular polysaccharides. Bar = 0.5 μm.

phosphate, S[0], and poly-β-hydroxybutyrate. When *M. bipunctata* is grown on media containing succinate or malate, refractile irregularly-shaped calcium oxalate inclusions (previously believed to be calcium carbonate) accumulate in the cells. In *M. bipunctata*, calcium oxalate is synthesized mainly via oxaloacetate conversion to acetate and oxalate, with the participation of oxaloacetate hydrolase in the TCA cycle (Grabovich et al., 1995). Depending on the concentration of organic compounds in the medium, oxalates may appear as ballast or serve as a pool of reserve organic matter that can maintain metabolism under unfavorable conditions. When used as ballast, the oxalates may be removed from the cells by oxidation to CO_2 using oxalate oxidase. The reaction is not associated with either biosynthetic or energy metabolism.

S[0] accumulates in cells grown in media containing FeS or CaS, but not in cells grown with thiosulfate. Sulfur globules also appear in cells that have been grown in the absence of sulfides and then incubated for 20 minutes in a 0.005–0.01% polysulfide solution. Electron micrographs of ultrathin sections show that the sulfur globules are located in the periplasm and within the invaginations formed by the cell membrane.

Colonies on solid media are non-pigmented, of a fine-grained structure, slightly opalescent, and flat, measuring 0.5–4 mm. Growth in static liquid occurs in the upper 3–5 mm layer.

M. bipunctata strains thus far isolated have been chemoorganotrophic, but they do not tolerate or utilize high concentrations of organic compounds. Low concentrations of growth substrates (5–10 mM) are apparently optimal. High concentrations induce rapid autolysis of cells due to hydrogen peroxide accumulation in the periplasm. The addition of sulfides, thiosulfate, or catalase to the growth medium increases the biomass yield by 20–40% and extends the survival of stored cultures from several days to a month. Acetate, succinate, malate, fumarate, oxalacetate, benzoate, and pyruvate are good substrates, but sugars, alcohols (with the exception of ethanol), and amino acids are not used. Ammonium salts and organic nitrogen compounds can serve as nitrogen sources. Vitamins are required for growth. Further physiological and biochemical characterization of *M. bipunctata* is shown in Table BXII.β.55.

Optimal temperature 28°C, optimal pH 7.5–8.2.

The complete TCA cycle functions in *M. bipunctata*, its only unusual feature being a low fumarate hydratase activity, leading to the accumulation of fumarate in the cells. The glyoxylate cycle also functions in *M. bipunctata*, regardless of the carbon source used. It has been suggested that the glyoxylate cycle compensates for the low fumarate hydratase activity, shunting fumarate to be converted to malate. Calcium oxalate formation and its intracellular accumulation have been shown to be another peculiarity of carbon metabolism in *M. bipunctata* (Grabovich et al., 1993).

M. bipunctata oxidizes sulfide to S[0], and thiosulfate to tetrathionate by means of hydrogen peroxide, a process that does not provide useful energy for the cells (Dubinina and Grabovich, 1984; Chekanova and Dubinina, 1990).

Isolated from the sediment in an aeration tank of a sewage treatment plant.

The mol% G + C of the DNA is: 67.6 (T_m).

Type strain: DSMZ 12705, VKM 1366.

TABLE BXII.β.55. Characterization of *Macromonas bipunctata* strains[a]

Characteristics	Reaction
Use as a sole carbon source:	
Organic acids:	
Acetate, benzoate, fumarate, malate, oxaloacetate, succinate	+
Citrate, isocitrate, aconitate, malonate, glycolate	−
α-Ketoglutarate, glyoxylate, lactate, formate, pyruvate, propionate, oxalate	d
Alcohols:	
Ethanol	d
Methanol, butanol, isobutanol, glycerol	−
Amino acids:	
Serine, aspartate, lysine, cysteine, tryptophan, alanine, histidine, phenylalanine, asparagine, methionine, proline, tyrosine	−
Peptone	−
Casein hydrolysate, yeast extract	+
Use as a sole nitrogen source:	
Ammonium	+
Peptone, casein hydrolysate	+
Nitrate and nitrite	−
Glutamate	+
Alanine, aspartate, serine, cysteine, cystine, methionine	d
Reduction of NO_3^- to NO_2^-	−
N_2 fixation	−
H_2S production from cysteine or $S_2O_3^{2-}$	−
S^0 production from sulfide	+
Hydrolysis of gelatin, starch, casein	−
Indole production	−
Catalase activity	+[b]
Oxidase activity	+
Urease activity	+
Anaerobic growth with nitrate, sulfate, thiosulfate, or fumarate as acceptors of electrons	−
Mol% G + C of DNA (T_m)	66–68

[a]Symbols: +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; d, 11–89% of the strains are positive.

[b]Activity of catalase is weak.

Genus VIII. **Polaromonas** *Irgens, Gosink and Staley 1996, 825*[VP]

JOHN J. GOSINK

Po'lar.o.mo'nas. M.L. adj. *polaris* pertaining to the geographic poles; Gr. fem. n. *monas* unit; M.L. fem. n. *Polaromonas* polar bacterium.

Cigar-shaped rods, 0.8 × 2.0–3.0 μm. Gram negative and encapsulated. Motile by a polar flagellum. Aerobic. Chemoorganotrophic, catalase and oxidase positive. Require amino acids, but not vitamins for growth. **Cellular fatty acids predominantly (74–79%) C$_{16:1 \, \omega 7c}$, and C$_{16:0}$ (14–17%), and some (7–9%) C$_{18:1 \, \omega 7c}$, C$_{18:1 \, \omega 9t}$, or C$_{18:1 \, \omega 12t}$. Organisms may contain gas vesicles.** Psychrophilic; growth temperature maximum of known strains is 15°C. 16S rDNA-based phylogeny shows this is a member of the *Comamonadaceae* in the *Betaproteobacteria*.

The mol% G + C of the DNA is: 52–57.

Type species: **Polaromonas vacuolata** Irgens, Gosink and Staley 1996, 825.

FURTHER DESCRIPTIVE INFORMATION

Five putative strains (34-P, 41-P, 54-P, JA, and JB) of *Polaromonas* have been isolated from Antarctic waters. Only one strain, 34-P, has been examined in depth by phenotypic, genotypic, and phylogenetic methods and hence officially recognized as a member of this genus.

Phylogenetic trees of *Polaromonas* and closely related taxa have been generated using distance, parsimony, and maximum likelihood methods. Analyses using various forms of parsimony (Swofford, 1991; Maddison and Maddison, 1992), with different character weighting masks and substitution matrix weights, give a number of different trees with similar structures (Fig.

BXII.β.40A). Distance (Fig. BXII.β.40B) and likelihood (Fig. BXII.β.40C) have also been performed on the data, using a transition to transversion ratio of 1.3 (Felsenstein, 1981, 1989; Olsen et al., 1994).

The results of these analyses show slightly different trees, depending on which assumptions for the model of evolution are used. All of these methods show that *Polaromonas* is a member of the *Betaproteobacteria* and shares a moderately close relationship with *Rhodoferax fermentans* (Hiraishi et al., 1991a), *Variovorax paradoxus*, (Davis et al., 1969; Willems et al., 1991a) and the environmental sequence "str. Stripa" (Ekendahl et al., 1994). Recent inspection of the Ribosomal Database Project (Maidak et al., 1999) small subunit database reveals additional partial "environmental population shotgun" sequences closely related to those of these organisms. More accurate or reproducible phylogenetic trees would be produced if full length sequences were available from the environmental clones. In addition, since these sequences have been obtained from uncultured bacteria, there are no phenotypic data available for the bacteria that produced these sequences, so comparison with these organisms can not proceed beyond the phylogenetic stage. The exact phylogenetic relationship among these taxa remains unclear pending future detailed examination.

Polaromonas strains appear as short, unicellular, Gram-negative rods (0.8 × 2–3 μm) that typically produce gas vacuoles, which
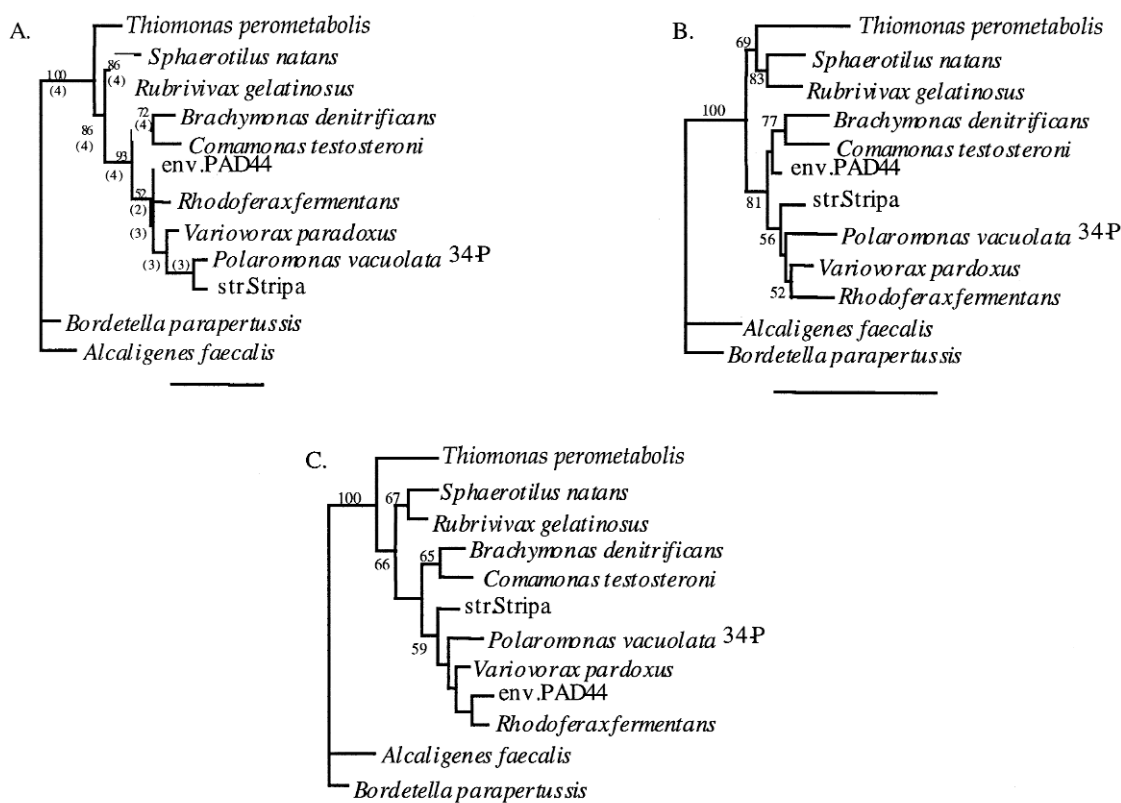
FIGURE BXII.β.40. The phylogenetic relatedness of *Polaromonas vacuolata* strain 34-P to the most closely related species. Numbers near the branches but not in parentheses indicate percent bootstrap support for that clade from 100 bootstrap resamplings. Only bootstrap values of 50% or greater are shown. Bars = 0.1 changes/average nucleotide position. A maximum parsimony tree (*A*), was determined using PAUP 3.0s by an exact (branch and bound) search, using a substitution matrix to correct for the various rates of nucleotide substitutions (Swofford, 1991; Maddison and Maddison, 1992). Numbers in parentheses near the branches indicate how many of the four equally most parsimonious trees share that branch structure. A neighbor joining tree (*B*) was made using PHYLIP 3.2 and a maximum likelihood tree (*C*) was made with fast DNAmL; both were made with a Kimura 2 parameter correction, using a transition to transversion ratio of 1.3 (Felsenstein 1989; Olsen et al., 1994).

appear as bright refractile areas within the cells. Gas vesicles are confirmed by transmission electron microscopy (Fig. BXII.β.41). Although cells are nonmotile under usual culture conditions, they produce polar flagella in addition to gas vacuoles (Fig. BXII.β.41). These bacteria produce circular, convex colonies with a smooth, glistening surface and an entire edge on agar plates. The colonies are chalky white in pigmentation. All strains produce large amounts (74–79%) of $C_{16:1\ \omega7c}$ and smaller amounts of $C_{16:0}$ (14–17%) (Irgens et al., 1996). A third fatty, $C_{18:1\ \omega7c}$, $C_{18:1\ \omega9v}$, $C_{18:1\ \omega12v}$, or possibly a combination of more than one of these, is present in lower amounts (7–9%). Such predominance of a single fatty acid is unusual in bacteria, and this is among the highest level of $C_{16:1\ \omega7c}$ in any bacterial species. Additional features are shown in Table BXII.β.56.

Although many members of the *Alphaproteobacteria* and *Gammaproteobacteria* have been shown to produce gas vesicles, this is the first described gas vacuolate member of the *Betaproteobacteria*, though there are likely many more gas vacuolate members among this phylogenetic group. Although gas vacuolate heterotrophic bacteria are well known inhabitants of aquatic ecosystems, until recently, none had been observed or isolated from marine habitats. In 1989, several types from Antarctica were reported growing in association with the sea ice microbial community (Irgens, et al., 1989; Staley et al., 1989). The ecological role of *Polaromonas* and the function that gas vesicles serve in that role are unknown.
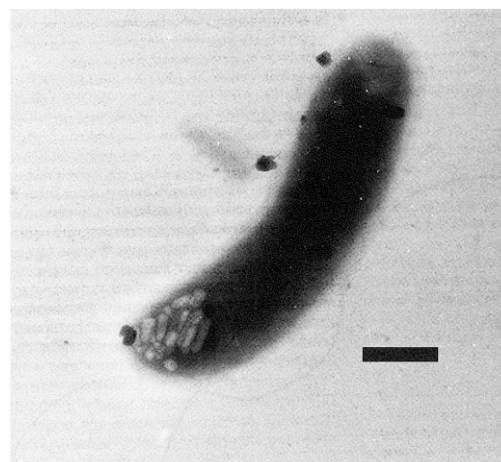

FIGURE BXII.β.41. An electron micrograph of *Polaromonas vacuolata* strain 34-P showing a cell containing several gas vesicles. Flagella are not attached. Bar = 0.5 µm. (Reprinted with permission from R.L. Irgens et al., International Journal of Systematic Bacteriology *46:* 822–26, 1996 ©International Union of Microbiological Societies.)

ENRICHMENT AND ISOLATION PROCEDURES

All strains were isolated from Antarctic waters near the U.S. Palmer Station, Anvers Island, Antarctica. These organisms are

TABLE BXII.β.56. Phenotypic and genotypic features of *Polaromonas* strains 34-P, 41-P, 54-P, JA, and JB [a,b]

Characteristic	*Polaromonas vacuolata* strain 34-P	*Polaromonas* sp. strain 41-P	*Polaromonas* sp. strain 54-P	*Polaromonas* sp. strain JA	*Polaromonas* sp. strain JB
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Urease	+	nd	nd	v	nd
Deaminase	+	−	−	−	−
Lipase	+	+	+	+	+
Agarase	−	nd	nd	nd	nd
Amylase, proteinase, tryptophanase, nitrate reductase, cysteine desulfurase	−	−	−	−	−
Lactate	+	+	+	+	+
Malate	+	+	−	+	−
Fumarate	+	−	−	−	−
Succinate	+	−	+	+	−
D-Glucose	+	−	−	−	+
Sucrose	−	−	−	−	+
Lactose	−	−	+	−	−
Ethanol	−	−	−	−	−
Acetate, pyruvate, propionate, citrate, oxaloacetate, butyrate, 2-oxoglutarate, glycerol, sorbitol	+	nd	nd	nd	nd
Formate, benzoate, malonate, maltose, D-fructose, D-xylose, D-ribose, cellobiose, D-mannose, L-fucose, melibiose, melezitose, L-rhamnose, sorbose, trehalose, methanol, erythritol, propanol	−	nd	nd	nd	nd
DL-Alanine	+	+	−	+	−
DL-Glutamate	+	+	+	+	−
DL-Aspartate	+	+	−	−	−
DL-Arginine	−	−	−	−	+
DL-Ornithine	−	−	−	−	−
DL-Proline, DL-asparagine	+	nd	nd	nd	nd
Glycine, DL-serine, DL-isoleucine, DL-lysine, DL-histidine, DL-methionine, DL-valine, DL-threonine, DL-tryptophan	−	nd	nd	nd	nd
Growth temperature range (°C)	≤ − 1.5–10	≤ − 1.5–7	≤ − 1.5–9	≤ − 1.5–9	≤ − 1.5–8
Growth salinity range, %	0–6	0.15–2.5	0.15–2.5	0.15–2.5	0.15–2.5
Vitamin requirement	none	nd	nd	nd	nd
Anaerobic growth	−	−	−	−	−
Mol% G + C	52	57	52	53	52

[a]For symbols see standard definitions; nd, not determined.

[b]Data from Irgens et al., 1989, 1996.

isolated by plating water samples onto SWCm (Staley et al., 1989) agar plates and incubating at 6°C for 6 weeks. Gas vacuolate colonies can be identified by their chalky appearance. *Polaromonas* strains are delineated from other gas vacuolate strains by their color, colony morphology, and fatty acid composition.

MAINTENANCE PROCEDURES

Stocks are maintained for general work on SWCm (Staley et al., 1989) at 4°C. Care must be taken when transferring or working with the strains to keep them and the growth media at 4°C. These cultures can be killed by leaving them out at room temperature for several hours or overnight. Long-term storage is best done by transferring cells to SWCm (Staley et al., 1989) broth with 25% glycerol or 10% DMSO and freezing at −80°C.

DIFFERENTIATION OF THE GENUS *POLAROMONAS* FROM OTHER GENERA

Analyses of 16S rDNA sequences using various phylogenetic methods produce slightly different trees. For all the trees, how-

ever, it is clear that strain 34-P is most closely related to *Rhodoferax fermentans* (Hiraishi et al., 1991a), *Variovorax paradoxus* (Davis et al., 1969; Willems et al., 1991a), "str. Stripa", and "env. PAD44" (Ueda, unpublished results). It is, however, phenotypically and genotypically quite different from these organisms. Strain 34-P is not photosynthetic and does not grow as a nonsulfur purple bacterium under conditions used for the growth of *R. fermentans*. Strain 34-P differs by 5% and 7% in 16S rDNA base homology from *V. paradoxus* (Davis et al., 1969; Willems et al., 1991a) and *R. fermentans* (Hiraishi et al., 1991a), respectively. Furthermore, other genotypic and phenotypic differences indicate marked differences among *Polaromonas vacuolata*, *V. paradoxus*, and *R. fermentans* (Table BXII.β.57). For example, the mol% G + C values are 52–57 versus 67–69 and 60, respectively (Davis et al., 1969; Irgens et al., 1989; Hiraishi et al., 1991a; Willems et al., 1991a). In addition, *V. paradoxus* and *R. fermentans* are both pigmented, are not gas vacuolate, and differ from *P. vacuolata* in cell shape and motility (Table BXII.β.57).

TABLE BXII.β.57. Phenotypic comparison of *Polaromonas vacuolata* to the two phylogenetically most closely related species[a]

Characteristic	Polaromonas vacuolata	Rhodoferax fermentans	Variovorax paradoxus
Shape of cells	rods	curved rods	straight or curved rods
Gas vesicles	+	−	−
Photosynthetic	−	+	−
Flagella:			
Peritrichous			+
Polar	+	+	
O₂ requirement:			
Facultative aerobe		+	
Obligate aerobe	+		+
Temperature relations:			
Mesophilic		+	+
Psychrophilic	+		
Colony pigmentation[b]	W	Pb	Y
Mol% G + C	52–57	60	67–69

[a]Data from Davis et al., 1969; Irgens et al., 1989; Hiraishi et al., 1991a.

[b]W, white; Pb, peach-brown; Y, yellow.

TAXONOMIC COMMENTS

The complete region of 16S rDNA phylogeny delineated by this genus remains uncertain pending nucleotide sequence analysis of additional members. In addition, the large number of partial nucleotide sequences closely related to the 16S rDNA sequence of this genus may blur resolution of this region of the phylogenetic tree.

List of species of the genus Polaromonas

1. **Polaromonas vacuolata** Irgens, Gosink and Staley 1996, 825[VP]

   va.cu.o.la′ ta. L. adj. *vacuus* empty; N.L. part. adj. *vacuolata* equipped with gas vacuoles.

   Cells contain gas vesicles. Temperature for optimum growth is 4°C, with a range of 0–12°C. Colonies are snowy white, circular, and convex, with a smooth surface and an entire edge. The more gas vesicles within the cells, the whiter the colony. Grows on fumarate, glucose, alanine, glutamate, and aspartate. Does not grow on sucrose, lactose, or arginine. There is good growth in media with NaCl concentrations ranging from 0–6.0% but no growth at 7.0%. Fatty acid composition is 75% $C_{16:1 \, \omega7c}$, 17% $C_{16:0}$, and 8% $C_{18:1 \, \omega7c}$, $C_{18:1 \, \omega9t}$, or $C_{18:1 \, \omega12t}$.

   The mol% G + C of the DNA is: 52.0 (T_m).

   Type strain: 34-P, ATCC 51984.

   GenBank accession number (16S rRNA): U14585.

Genus IX. **Rhodoferax** *Hiraishi, Hoshino, and Satoh 1992a, 192[VP] (Effective publication: Hiraishi, Hoshino, and Satoh 1991a, 334)*

AKIRA HIRAISHI AND JOHANNES F. IMHOFF

Rho.do.fe′ rax. Gr. n. *rhodon* rose; L. adj. *ferax* fertile; M.L. masc. n. *Rhodoferax* red and fertile.

Cells are curved or vibrioid rods, motile by means of polar flagella, that multiply by binary fission and do not form endospores and capsules. **Gram negative, belonging to the *Betaproteobacteria.*** Internal membrane systems are poorly developed or absent. **Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the spheroidene series. Contain ubiquinones and rhodoquinones with eight isoprene units (Q-8 and RQ-8). Straight-chain $C_{16:1}$ and $C_{16:0}$ acids are the major components of cellular fatty acids. $C_{8:0 \, 3OH}$ acid is present.** Membrane-bound fumarate reductase activity occurs with $FMNH_2$ as the electron donor.

   Growth is possible by photosynthesis, aerobic respiration, or fermentation. Photoheterotrophy with various organic compounds as carbon sources is the preferred mode of growth. Cells grow well with simple organic compounds as electron donors and carbon sources and in complex media containing peptone, yeast extract, or Casamino acids. **Mesophilic and psychrophilic, neutrophilic fresh water bacteria.** Habitat: ditchwater, activated sludge, Antarctic microbial mats.

   The mol% G + C of the DNA is: 59.8–61.5.

   Type species: **Rhodoferax fermentans** Hiraishi, Hoshino and Satoh 1992a, 192 (Effective publication: Hiraishi, Hoshino and Satoh 1991a, 334.)

FURTHER DESCRIPTIVE INFORMATION

According to 16S rDNA sequences, the closest phylogenetic relative to the *Rhodoferax* species is the purely chemotrophic *Variovorax paradoxus* (sequence similarity of 96%). The nearest phototrophic phylogenetic neighbor is *Rubrivivax gelatinosus* (91% sequence similarity; see Fig. 4 [p.132] of the introductory chapter "Anoxygenic Phototrophic Purple Bacteria", Volume 2, Part A).

In addition, the aerobic, bacteriochlorophyll-containing bacterium *Roseateles depolymerans* is within the same phylogenetic group.

R. fermentans is a facultatively photoheterotrophic bacterium that grows anaerobically in the light and aerobically in darkness at full atmospheric oxygen tension and exhibits a doubling time of 3–4 h under optimal growth conditions. Colonies grown on agar media are of intermediate size (2–4 mm after one week of incubation), round, smooth, circular, convex, and peach brown. However, rigid colonies frequently occur upon subculture. Phototrophic liquid cultures become peach brown, whereas aerobic, chemotrophic cultures are colorless or faintly pink. Absorption spectra of phototrophically grown cells or membrane preparations show major absorption maxima at around 800 and 850 nm, indicating that the cells contain the core light-harvesting complex (LH I), together with the photosynthetic reaction center and the peripheral light-harvesting complex (LH II).

One of the most remarkable properties of *Rhodoferax fermentans* is its capability of anaerobic fermentation in darkness. In Hugh-Leifson's OF test, which is commonly used to characterize chemoorganotrophic bacteria, *R. fermentans* produces acid from glucose in both open and sealed tubes within a few days of incubation (Hiraishi and Kitamura, 1984). Such a rapid glucose fermentation has not been found in other species of phototrophic bacteria described so far. Fermentative growth in darkness occurs on pyruvate and sugars, among which fructose is the best substrate. The addition of bicarbonate significantly enhances anaerobic growth in the dark. The doubling time for fermentative growth on 20 mM fructose plus 30 mM bicarbonate is 4–5 h (Hiraishi, 1988b). The end products of fructose fermentation are acetate, formate, lactate, succinate, and ethanol. Hypophosphite, a potent inhibitor of pyruvate–formate lyase, completely suppresses the production of formate and increases the amount of succinate excreted. These observations suggest that the bicarbonate-dependent fermentative growth may be linked to CO_2 fixation via part of the reductive TCA cycle and the subsequent reduction of fumarate to succinate. GDP-dependent phosphoenolpyruvate carboxykinase has been suggested to function as a key enzyme for CO_2 fixation (Hiraishi, 1988b), and reduced FMN-linked fumarate reductase activity that is possibly associated with a low-potential rhodoquinone within the cells has been found in the membrane preparations (Hiraishi, 1988a).

While many species of phototrophic purple nonsulfur bacteria exhibit anaerobic growth in darkness coupled with reduction of trimethylamine-*N*-oxide or dimethylsulfoxide as the terminal electron acceptor, *R. fermentans* lacks these properties. Most strains of this species are also devoid of nitrate reductase activity, although strain DSM 10139 is highly active at nitrate reduction (Hougardy and Klemme, 1995).

Some information has been available on the components of the respiratory and photosynthetic electron transport systems of *R. fermentans*. Cells produce two quinone molecules, Q-8 and RQ-8, constitutively. The RQ/Q molar ratio is 0.5–0.7 under phototrophic growth conditions with malate as the carbon source (Hiraishi and Hoshino, 1984). This ratio is higher (0.9–1.7) in cells grown fermentatively or phototrophically and becomes much lower (0.1–0.2) in aerobically grown cells (Hiraishi et al., 1991a). It has been shown that the oxidative electron transport chain is branched at the ubiquinone level and does not involve rhodoquinone (Hochkoeppler et al., 1995b). Aerobically grown cells contain four *b*-type and three *c*-type membrane-bound cytochromes but lack soluble *c*-type cytochromes. A soluble, high-potential iron-sulfur protein (HiPIP) functions as an alternative

to the soluble cytochrome *c* in linking the bc_1 complex to the terminal oxidase in respiratory electron transfer (Hochkoeppler et al., 1995a). Phototrophic cells contain the reaction center complex with tetraheme *c* as direct electron donors to the bacteriochlorophyll dimer. HiPIP is also competent as an alternative to the soluble *c*-type cytochrome in photosynthetic electron transfer (Hochkoeppler et al., 1995a, 1996).

The genes (*puf*) coding for the L and M protein subunits of the photosynthetic reaction center have been analyzed by PCR cloning (Nagashima et al., 1997a). The deduced amino acid sequences of the puf proteins of *R. fermentans* are most similar to those of *Rubrivivax gelatinosus* (77% identity). A phylogenetic analysis based on the nucleotide sequences of DNA fragments coding for the L and M subunits has shown that the sequences of *R. fermentans* and of two other species of the *Betaproteobacteria*, *Rubrivivax gelatinosus* and *Rhodocyclus tenuis*, are positioned among those of *Alphaproteobacteria*. This contrasts with their phylogenetic relations, as based on 16S rDNA sequences (see Fig. 4 [p. 132] in the introductory chapter "Anoxygenic Phototrophic Purple Bacteria", Volume 2, Part A). The inconsistency is explained by possible horizontal transfer of the genes encoding the reaction center during evolution of photosynthesis.

As with other phototrophic *Betaproteobacteria*, the major phospholipid components of *Rhodoferax fermentans* are cardiolipin, phosphatidylethanolamine, and phosphatidylglycerol (A. Hiraishi, unpublished data). Straight-chain $C_{16:1}$ and $C_{16:0}$ acids are the main components of cellular fatty acids (see Table BXII.β.58).

Rhodoferax antarcticus is characterized by its preference for low temperatures and grows optimally at 12–18°C, but not above 25°C (Madigan et al., 2000b).

ENRICHMENT AND ISOLATION PROCEDURES

The natural habitats of *Rhodoferax* species are freshwater environments that are rich in organic matter. Sulfide-rich water bodies may not provide favorable conditions for *R. fermentans* because of its inability to use sulfide as the electron donor for growth and its weak tolerance toward sulfide. Strains of *Rhodoferax fermentans* have been isolated from pond water, sewage, and activated sludge. *R. antarcticus* was isolated from an Antarctic microbial mat.

Enrichment and isolation are possible under conditions also suitable for most of the species of phototrophic purple nonsulfur bacteria. Although it is not easy to perform selective enrichment of *Rhodoferax fermentans* from environmental samples, the addition of 0.5 mM EDTA to the enrichment medium may be effective for suppressing the overgrowth of possibly co-existing, fast growing phototrophic species, such as *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* (Hougardy and Klemme, 1995). A suitable enrichment medium consists of basal mineral medium, one or more simple organic compounds (e.g., 0.1% acetate or 0.2% glucose), and a vitamin mixture or 0.01% yeast extract. Incubation is under anoxic conditions under incandescent illumination at 1000–2000 lux at 28°C for *R. fermentans* or at 12–18°C for *R. antarcticus*.

A simple medium for growth and purification of *Rhodoferax* is MYCA medium (Hiraishi et al., 1991a), which contains 0.1% DL-malate, 0.3% yeast extract, 0.2% Casamino acids, and 0.05% $(NH_4)_2SO_4$ and is adjusted to pH 6.6–6.8.

MAINTENANCE PROCEDURES

Cultures are well-preserved in liquid nitrogen or by lyophilization. Preservation is also possible in a mechanical freezer at −80°C.

TABLE BXII.β.58. Differential characteristics of anoxygenic phototrophic *Betaproteobacteria* of the order *Burkholderiales*: genera *Rhodoferax* and *Rubrivivax*[a]

Characteristic	*Rhodoferax fermentans*	*Rhodoferax antarcticus*	*Rubrivivax gelatinosus*
Cell diameter (μm)	0.6–0.9	0.7	0.4–0.7
Cell shape	Curved rods	Curved rods	Straight to curved rods
Motility	+	+	+
Slime production	−	−	+
Color	Peach brown	Peach brown	Brown
Major carotenoids	Spheroidene, OH-spheroidene, spirilloxanthin	Most likely spheroidene and OH-spheroidene	Spheroidene, OH-spheroidene, spirilloxanthin
Growth factors	thiamine, biotin	biotin	thiamine, biotin[b]
Gelatin liquefaction	+	nd	+
Fructose fermentation	+	−	−
Starch hydrolysis	−	nd	+
Tween 80 lysis	−	nd	+
Carbon sources:			
Benzoate	−	−	−
C_{10} to C_{18} fatty acids	nd	nd	+
Citrate	+	+	+
Mannitol	+	−	−
Sorbitol	+	nd	+
N_2-fixation	+	+	+
Fumarate reductase activity:			
With reduced methylviologen	Low	nd	High
With $FMNH_2$	High	nd	Low
Major fatty acids:			
$C_{16:0}$	33–39	nd	24–35
$C_{16:1}$	52–54	nd	35–45
$C_{18:0}$	<1	nd	1–3
$C_{18:1}$	5	nd	16–25
3-OH fatty acid	8:0	nd	10:0
Major quinones	Q-8 + RQ-8	nd	Q-8 + MK-8
Mol% G + C of DNA			
by HPLC	59.8–60.3	nd	71.2–72.1
by Bd	nd	nd	70.5–72.4
by T_m	nd	61.5	70.2–71.9

[a]Symbols: +, positive in most strains; −, negative in most strains; Q-8, ubiquinone - 8; RQ-8, rhodoquinone-8; MK-8, menaquinone-8.

[b]Some strains may also require pantothenate.

DIFFERENTIATION OF THE GENUS *RHODOFERAX* FROM OTHER GENERA

The genus *Rhodoferax* is differentiated from other genera of phototrophic *Proteobacteria* by its phylogenetic position and its unique physiological and chemotaxonomic properties. The characteristic features of *Rhodoferax* include fructose fermentation, Q-8 and RQ-8 production, and relatively low mol% G + C contents of the DNA. Differential characteristics of *Rhodoferax* species and other phototrophic *Betaproteobacteria* are given in Tables 6 (p. 130) and 7 (p. 131) of the introductory chapter "Anoxygenic Phototrophic Purple Bacteria", Volume 2, Part A. The phylogenetic relationships of the phototrophic *Betaproteobacteria* are shown in Fig. 4 (p. 132) of the introductory chapter "Anoxygenic Phototrophic Purple Bacteria", Volume 2, Part A. *Rhodoferax* is compared to *Rubrivivax gelatinosus* in Tables BXII.β.58 and BXII.β.59.

TAXONOMIC COMMENTS

Strains of *R. fermentans* were first recognized as the "*Rhodocyclus gelatinosus*-like (RGL)" group (Hiraishi and Hoshino, 1984), because they were phenotypically similar to *Rubrivivax gelatinosus* (at that time known as *Rhodocyclus gelatinosus*), in particular appearing to resemble *Rubrivivax gelatinosus* subgroup II (Weckesser et al., 1969). More detailed physiological, chemotaxonomic, and genetic studies revealed major differences between the RGL group and *Rubrivivax gelatinosus*, and these observations led to the proposal for the classification of the RGL group into the new genus *Rhodoferax* (Hiraishi et al., 1991a) as *R. fermentans*. Later, a phylogenetic study based on 16S rDNA sequences showed that the genera *Rhodoferax*, *Rubrivivax*, and *Rhodocyclus* are phylogenetically distinct groups within the *Betaproteobacteria* (Hiraishi, 1994).

List of species of the genus Rhodoferax

1. **Rhodoferax fermentans** Hiraishi, Hoshino and Satoh 1992a, 192[VP] (Effective publication: Hiraishi, Hoshino and Satoh 1991a, 334.)

fer.men′ tans. M.L. part. adj. *fermentans* fermenting.

Cells are curved or vibrioid rods, occurring singly or in

TABLE BXII.β.59. Carbon sources and electron donors used by anoxygenic phototrophic *Betaproteobacteria* of the order *Burkholderiales*: genera *Rhodoferax* and *Rubrivivax*[a]

Source/donor	*Rhodoferax fermentans*	*Rhodoferax antarcticus*	*Rubrivivax gelatinosus*
Carbon source			
Acetate	+	+	+
Arginine	−	nd	nd
Aspartate	+	+	+
Benzoate	−	−	−
Butyrate	+	+	+/−
Caproate	−	−	nd
Caprylate	−	−	nd
Citrate	−	+	+
Ethanol	+/−	−	+
Formate	−	−	+/−
Fructose	+	+	+
Fumarate	+	+	+
Glucose	+	+	+
Glutamate	+	−	+
Glycerol	−	−	−
Glycolate	−	−	nd
Lactate	+/−	+	+
Malate	+	+	+
Malonate	−	nd	nd
Mannitol	+	−	−
Mannose	+	−	+
Methanol	−	−	+/−
Pelargonate	nd	nd	nd
Propionate	−	−	+/−
Pyruvate	+	+	+
Sorbitol	+	nd	−
Succinate	+	+	+
Tartrate	−	nd	+/−
Valerate	nd	−	+
Electron donor:			
Hydrogen	nd	+	+
Sulfide	−	−	−
Sulfur	nd	−	−
Thiosulfate	−	−	−

[a] Symbols: +, positive in most strains; −, negative in most strains; +/− variable in different strains; nd, not determined.

pairs (Fig. BXII.β.42), 0.6–0.9 × 2–5 μm under optimal growth conditions. Long chains and helical filaments may be produced, especially when cells are grown with sugars under phototrophic or fermentative growth conditions. Spheroplast-like coccoid cells occasionally occur as cultures age (see Fig. BXII.β.42). **Motile by single polar flagella** (Fig. BXII.β.43). Internal membrane systems are poorly developed or absent (Fig. BXII.β.44). The color of photosynthetic cultures and colonies is peach brown. Absorption spectra of living cells show maxima at 377–378, 457–458, 484–485, 589–590, 799–801, and 850–851 nm. **Photosynthetic pigments are bacteriochlorophyll *a* esterified with phytol and carotenoids of the spheroidene series** with spheroidene, spirilloxanthin, and OH-spheroidene are present as major components.

Photoheterotrophic growth under anoxic conditions in the light or chemotrophic growth under oxic conditions in the dark at full atmospheric oxygen tension is possible. Photoorganotrophy with sugars, peptone, yeast extract, and Casamino acids as electron donors and carbon sources is the preferred mode of growth. In addition, simple organic compounds, such as acetate and intermediates of the tricarboxylic acid cycle, also support growth as carbon sources. The carbon sources utilized are listed in Table BXII.β.59.

Also utilized are xylose, arabinose, galactose, gluconate, and asparagine. Bicarbonate-dependent dark fermentative growth is also possible. Sulfide, thiosulfate, and other reduced sulfur compounds do not serve as electron donors for photoautotrophic growth. Sulfide tolerance is weak; only 0.2 mM sulfide inhibits growth in the presence of 0.05% yeast extract. Ammonium salts and glutamate, but not nitrate, serve as nitrogen sources. The capacity for nitrogen fixation appears to be present, as suggested by the observation that nitrogenase-dependent H_2 production occurs in an ammonium-deficient medium. Sulfate is assimilated as a sulfur source. Catalase is negative or weakly positive. Oxidase reaction is positive. Proteolytic activities are present, but weak compared to those of the phylogenetic relative *Rubrivivax gelatinosus*. Gelatin is weakly hydrolyzed. Thiamine and biotin are required as growth factors.

Mesophilic and neutrophilic fresh water bacterium with optimal growth at 25–30°C (no growth at 37°C) and pH 6.5–7.0 (pH-range: pH 5–9). No growth occurs in the presence of 1.5% NaCl. Habitat: freshwater ponds, sewage ditches, and activated sludge. Major quinone components are Q-8 and RQ-8.

The mol% G + C of the DNA is: 59.8–60.3 (HPLC); type strain, 60.1 (HPLC).

Type strain: FR2, ATCC 49787, DSM 10138, JCM 7819.

GenBank accession number (16S rRNA): D16211.

2. **Rhodoferax antarcticus** Madigan, Jung, Woese, Achenbach 2000b, 275[VP]

ant.arc′ ti.cus. M.L. adj. *antarcticus* belonging to, coming from Antarctica.

Cells are curved or vibrioid rods, 0.7 × 2–3 μm under optimal growth conditions. Highly motile by polar flagella. Internal membrane systems are poorly developed or absent. The color of photosynthetic cultures and colonies is peach brown. Absorption spectra of living cells show maxima at 427, 452, 487, 518, 582, 799, 819, and 866 nm. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids, most likely of the spheroidene series.

Photoheterotrophic growth under anoxic conditions in the light or chemotrophic growth under oxic conditions in the dark at full atmospheric oxygen tension is possible. Acetate pyruvate, lactate, succinate, malate, fumarate, glucose, fructose, sucrose, citrate, and aspartate support growth as carbon sources. Not utilized are methanol, ethanol, propanol, butanol, mannitol, glycerol, lactose, benzoate, formate, propionate, butyrate, valerate, caproate, heptanoate, glutamate, glutamine, and oxoglutarate. Bicarbonate-dependent dark fermentative growth with glucose and fructose is not possible. Sulfide at 2 mM inhibits growth. Photoautotrophic growth with hydrogen is slow. Ammonium salts, glutamate, glutamine, and aspartate serve as nitrogen sources. The capacity for dinitrogen fixation is present. Sulfate is assimilated as a sulfur source. Biotin is required as growth factor.

Psychrophilic and neutrophilic freshwater bacterium with optimal growth at 12–18°C (range: 0–25°C, no growth above 25°C). NaCl is not required and growth inhibitory above 2%.

Habitat: Antarctic microbial mat.

The mol% G + C of the DNA is: 61.5 (T_m).

Type strain: ATCC 700587.

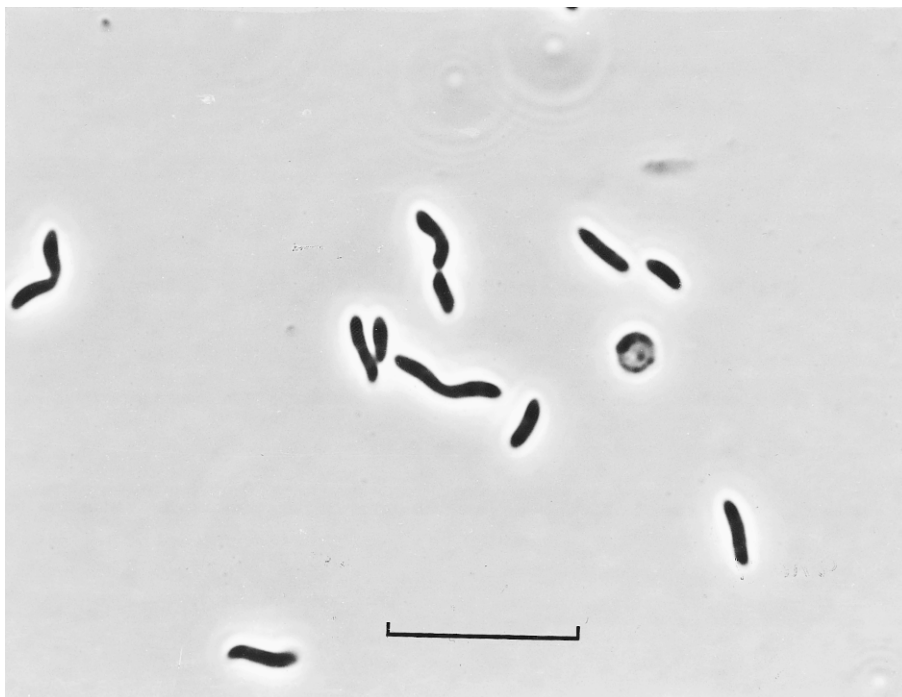*GenBank accession number (16S rRNA)*: AF084947.

FIGURE BXII.β.42. Phase-contrast micrograph showing cell morphology of *Rhodoferax fermentans* (strain FR2). Curved rods occurring singly and in pairs and a spheroplast-like cell are observed. Bar = 10 μm.
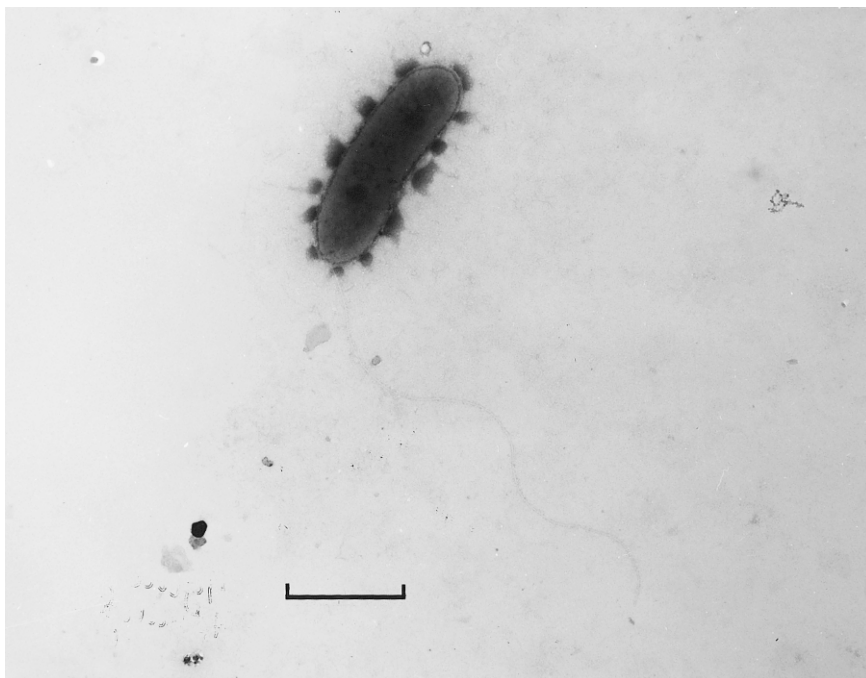

FIGURE BXII.β.43. Electron micrograph of a negatively stained cell of *Rhodoferax fermentans* (strain FR2) with a polar flagellum. Bar = 1 μm.
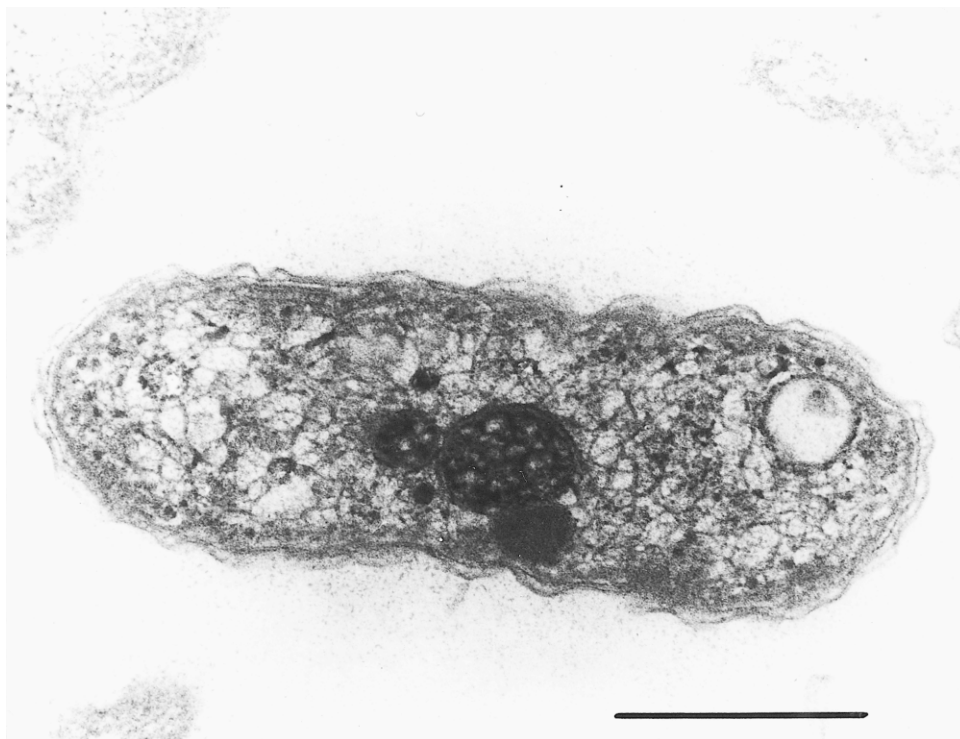
FIGURE BXII.β.44. Thin-section electron micrograph showing ultrastructure of *Rhodoferax fermentans* (strain FR2) grown phototrophically. Bar = 0.5 μm.

Genus X. **Variovorax** Willems, De Ley, Gillis and Kersters 1991a, 446[VP]

ANNE WILLEMS, JORIS MERGAERT AND JEAN SWINGS

Va.ri.o' vo.rax. L. adj. *varius* various; L. adj. *vorax* voracious; M.L. masc. n. *Variovorax* (bacteria) devouring a variety (of substrates).

Straight to slightly curved rods, 0.5–0.6 × 1.2–3.0 μm, occurring singly or in pairs. **Gram negative. Motile by means of sparse, peritrichous flagella**. Colonies are yellow due to the presence of carotenoid pigments. **Aerobic, having a strictly respiratory type of metabolism with O$_2$ as the terminal electron acceptor. Oxidase and catalase positive. Chemoorganotrophic. Some strains are capable of lithoautotrophic growth**, using H$_2$ as an energy source. These are referred to as biotype I, whereas nonlithoautotrophic strains are referred to as biotype II (Davis et al., 1969). Good growth occurs on media containing carbohydrates, organic acids, amino acids, or peptone.

The mol% G + C of the DNA is: 66.8–69.4.

Type species: **Variovorax paradoxus** (Davis 1969) Willems, De Ley, Gillis and Kersters 1991a, 447 (*Alcaligenes paradoxus* Davis *in* Davis, Doudoroff, Stanier and Mandel 1969, 387.)

FURTHER DESCRIPTIVE INFORMATION

Flagellation In a study of the cell morphology and flagellation of Gram-negative hydrogen bacteria, Aragno et al. (1977) defined three types of flagella based on electron microscopy. They reported that *V. paradoxus* cells have one to three laterally inserted type II flagella. This oligotrichous flagellation was originally described as "degenerately peritrichous" (Davis et al., 1969). The flagella have diameters of 15–17 nm. Flagellar wave-

lengths have been observed to be of two distinct types, one about half as long (1.2–1.3 μm) as the other (2.2–2.5 μm) occurring in the same culture (Aragno et al., 1977). Pili are inserted at the polar caps (Aragno et al., 1977).

Cell wall composition Walther-Mauruschat et al. (1977) have studied the cell envelopes of Gram-negative hydrogen bacteria and distinguished three types of cell walls, differing mainly in visibility and location of the peptidoglycan layer. *Variovorax* possesses type I cell walls, typical of most Gram-negative bacteria and characterized by a multilayered structure, consisting of an outer membrane and a cytoplasmic membrane of similar dimensions and appearance, which are separated by a dense layer of peptidoglycan.

Ultrastructure Intracellular mesosome-like membrane systems with a spiral appearance are present and often located in the area of cell division or at the cell poles (Walther-Mauruschat et al., 1977). Their significance is unclear, and they are now regarded mainly as artifacts resulting from the preparation of cells for electron microscopy. Intracellular inclusions of poly-3-hydroxybutyrate and polyphosphate are present, and translucent, glycogen-like inclusions may also be detected (Walther-Mauruschat et al., 1977). Cytochromes of the *a*, *b*, and *c* types are present, including a cytochrome *a2* with a peak at 625 nm in the reduced/oxidized spectrum (Davis et al., 1969).

Colonial characteristics Colonies on nutrient agar are glistening and shiny, yellow or greenish yellow. Occasionally, two types of colonies may occur in a single culture. Often, these types prove to be unstable and cannot be separated. Sometimes, non-mucoid variants may be separated from mucoid strains, but SDS–polyacrylamide electrophoregrams of whole-cell proteins of such types are always identical. The yellow pigments are carotenoids with absorption maxima in acetone of approximately 405 and/or 425 nm (Davis et al., 1969). More recently, Urakami et al. (1995a) have reported an absorption maximum of 420 nm for the carotenoid pigments of *V. paradoxus* DSM 30034^T.

Nutrition and metabolism A large variety of organic compounds may be used as sole carbon sources (Table BXII.β.60). Of a total of 143 organic compounds tested, 99 are used by *V. paradoxus* strains (Davis et al., 1969). No growth factors are required. Two biotypes are recognized. Strains of biotype I are able to grow autotrophically using the aerobic oxidation of hydrogen as an energy source. The hydrogenase is membrane-bound and does not reduce NAD (Schneider and Schlegel, 1977). It is similar to that of *Ralstonia eutropha* in that PCR tests with primers specific for the *R. eutropha* hydrogenase gene yield products of the correct size for *V. paradoxus* but no product for other hydrogen bacteria (Lechner and Conrad, 1997). No soluble hydrogenase is present (Schneider and Schlegel, 1977). Strains of biotype II cannot grow autotrophically or oxidize hydrogen (Davis et al., 1969).

Nitrate, but not atmospheric nitrogen, can be used by many strains as a nitrogen source. *Meta-* and *para-*hydroxybenzoate are metabolized via the *ortho* cleavage of protocatechuate. A new pathway for the degradation of homovanillic acid, distinct from that of *Pseudomonas putida*, has been reported for a *V. paradoxus* strain isolated from farmyard soil by enrichment on vanillylpyruvate. Homovanillic acid is degraded via ring hydroxylation to a dihydroxymonomethoxyphenylacetic acid, which is then cleaved by homogentisate 1,2-dioxygenase (Allison et al., 1995). Catechol 1,2-dioxygenase from *V. paradoxus* has been purified and characterized (Matevosyan et al., 1989).

Biosynthesis of aromatic amino acids *V. paradoxus* strains possess prephenate dehydrogenase and arogenate dehydrogenase that are reactive with either NAD or NADP. Arogenate dehydratase activity is absent; prephenate dehydratase is present, and its activity is increased 2–3-fold by 0.5 mM L-tyrosine (Byng et al., 1983). 3-Deoxy-D-arabinoheptulosonate 7-phosphate synthetase is inhibited by phenylalanine and chorismate (Whitaker et al., 1981). The various pathways for the biosynthesis of aromatic amino acids and their regulation have been shown to be useful indicators for the differentiation of different groups of pseudomonad bacteria (Byng et al., 1983).

Chemotaxonomic characteristics *Variovorax* possesses a Q-8 ubiquinone system (Urakami et al., 1995a). Major fatty acids (representing at least 5% of total fatty acids) are palmitic acid ($C_{16:0}$), a cyclopropane substituted fatty acid ($C_{17:0\ cyclo}$), *cis-*vaccenic acid ($C_{18:1}$), palmitoleic acid ($C_{16:1}$), 3-hydroxydecanoic acid ($C_{10:0\ 3OH}$), and 2-hydroxytetradecanoic acid ($C_{14:0\ 2OH}$). (Willems et al., 1989; Urakami et al., 1995a). Characteristic polyamines are putrescine and 2-hydroxyputrescine (Busse and Auling, 1988).

Plasmids Plasmid DNA is regarded as particularly important for the genetic adaptation of microorganisms in the natural environment, because as a mobile form of DNA, it can provide new phenotypes, allowing bacteria to survive and even thrive under changing and often adverse environmental conditions (Leahy and Colwell, 1990). Pathways for the degradation of various complex organic molecules have been shown to be plasmid encoded in *Pseudomonas*-like bacteria (Chakrabarty, 1976). Since *V. para-*

TABLE BXII.β.60. Characteristics of *Variovorax paradoxus*[a,b]

Characteristic	Reaction
Oxidase, catalase, urease	+
Denitrification	−
Nitrite reduction	−
Intracellular accumulation of poly-3-hydroxybutyrate	+
Extracellular hydrolysis of poly-3-hydroxybutyrate, Tween 80	+
Hydrolysis of starch, gelatin	−
Growth with D-glucose, D-fructose, D-galactose, L-arabinose, D-fucose, D-xylose, D-mannose, sorbitol, D-mannitol, D-arabitol, ethanol, glycerol, D,L-glycerate, citrate, mesaconate, succinate, fumarate, D,L-lactate, L-malate, D,L-3-hydroxybutyrate, α-ketoglutarate, D-gluconate, 5-ketogluconate, acetate, pyruvate, sebacate, mesaconate, pantothenate, adipate, pimelate, hydroxymethylglutarate, quinate, *m*-hydroxybenzoate, *p*-hydroxy-benzoate, L-leucine, L-histidine, L-α-alanine, L-glutamate, L-proline, L-aspartate, L-phenylalanine, L-ornithine, D,L-4-aminobutyrate[c]	+
Growth with L-xylose, L-sorbose, D-melibiose, sucrose, trehalose, melezitose, D-raffinose, maltose, lactose, D-turanose, D-tagatose, β-gentiobiose, α-methylxyloside, α-methyl-D-mannoside, α-methyl-D-glucoside, amygdalin, arbutin, esculin, salicin, inulin, starch, glycogen, dulcitol, *meso*-erythritol, heptanoate, caprylate, pelargonate, caprate, oxalate, anthranilate, nicotinate, testosterone, benzoate, *o*-hydroxybenzoate, benzylamine, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, D-mandelate, isophthalate, terephthalate, L-valine, L-lysine, L-norleucine, L-cysteine, L-methionine, D-tryptophan, L-citrulline, L-arginine, D,L-kynurenine, D,L-2-amino-butyrate, δ-aminovalerate, trigonellin, urea, amylamine, ethylamine, diaminobutane, glucosamine, putrescine, spermine, histamine, tryptamine, betaine, sarcosine, creatine, acetamide, *N*-acetylglucosamine[c,d]	−
Utilization of D-fucose, L-fucose, cellobiose, D-ribose, L-rhamnose, D-arabinose, D-lyxose, adonitol, *meso*-inositol, xylitol, L-arabitol, 2-ketogluconate, saccharate, malonate, suberate, D-malate, *meso*-tartrate, D-tartrate, L-tartrate, mucate, glycolate, propionate, butyrate, isobutyrate, valerate, isovalerate, malonate, caproate, maleate, glutarate, azelate, levulinate, aconitate, citraconate, itaconate, L-mandelate, phthalate, phenylacetate, kynurenate, 2,3-butylene glycol, phenol, D-α-alanine, β-alanine, L-tryptophan, L-serine, L-threonine, L-isoleucine, L-tyrosine, L-tryptophan, D,L-norvaline, D,L-3-aminobutyrate, butylamine, pentylamine, glycine, ethanolamine[d,e]	d
Susceptible to:	
Chloramphenicol (30 μg/disk), tetracycline (30 μg/disk), kanamycin (30 μg/disk), neomycin (30 μg/disk), polymyxin B (300 U/disk)	+
Erythromycin (15 μg/disk), streptomycin (10 μg/disk)	d
Ampicillin (10μg/disk), methicillin (5 μg/disk), novobiocin (30μg/disk)	−

[a]For symbols see standard definitions.

[b]Data taken from Davis et al. (1969, 1970) (14 strains), Auling et al. (1978), Kersters and De Ley (1984b) (14 strains), and Aragno and Schlegel (1992).

[c]Aragno and Schlegel (1992) reported 11–89% of strains grew on citrate, acetate, *p*-hydroxybenzoate, L-leucine, L-histidine, L-α-alanine, L-glutamate, L-aspartate, L-phenylalanine, L-valine, and δ-aminovalerate in API auxanographic galleries.

[d]Aragno and Schlegel (1992) reported all strains grew on oxalate, 2–ketoglutarate, and glutarate in API auxanographic galleries.

[e]Aragno and Schlegel (1992) reported all strains failed to grow on cellobiose, *meso*-inositol, butylamine, and ethanolamine in API auxanographic galleries.

doxus strains are soil and water bacteria, it is not surprising that various plasmids have been reported to occur. Strains isolated from soil samples contaminated with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) contain plasmids pJP2, which carries genes for the degradation of 2,4-D, and pJP3, which carries genes conferring resistance to merbromin, phenyl-mercury acetate, and mercuric ions, and the ability to degrade *m*-chlorobenzoate (Don and Pemberton, 1981). Another 2,4-D-degradative plasmid has been isolated from a *V. paradoxus* strain from agricultural soil and is capable of integrating in the chromosome without loss of the 2,4-D$^+$ phenotype. In the same study, a very similar plasmid pKA4 was found in a 2,4-D-degrading *Ralstonia pickettii* strain, suggesting natural horizontal gene transfer among genera (Ka and Tiedje, 1994). This has been supported by use of *V. paradoxus* as the recipient organism for the large catabolic plasmid pJP4 of *Ralstonia eutropha* in a study of horizontal gene transfer in soil (Neilson et al., 1994).

Di Giovanni et al. (1996) have observed that several different plasmids may occur per strain in a study of 32 strains of 2,4-D-degrading *V. paradoxus* isolates from a sample of contaminated soil. Based on the size and number of plasmids, they have distinguished 6 groups among the strains, with each group containing identical and unique plasmids of diverse size. Curing of the plasmids results in the loss of the ability to break down 2,4-D. Comparison of restriction patterns of plasmids from 2,4-D$^+$ and 2,4-D$^-$ strains shows that plasmids involved in 2,4-D-degradation in different strains can have different origins. The data suggest that plasmids are frequently exchanged within a population, enhancing gene transfer and recombination events (Di Giovanni et al., 1996).

Hydrogen metabolism and autotrophic growth are thought to be, at least in part, plasmid-linked, because exposure of a *V. paradoxus* strain to the plasmid-curing agent mitomycin C results in the loss of the autotrophic phenotype (Lim et al., 1980).

Antibiotic sensitivity Auling et al. (1978) have studied the susceptibility to antibiotics of five *V. paradoxus* strains and reported all strains to be sensitive to five of the ten antibiotics tested (see Table BXII.β.60).

Ecology *Variovorax* is a common soil inhabitant, also occurring in contaminated soils, where it may harbor plasmids carrying genes involved in the breakdown of complex chemicals and resistance to toxic compounds. *Variovorax* strains dominate the 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading population in agricultural soil amended with this herbicide (Dunbar et al., 1995). A strain of *V. paradoxus* capable of degrading 2,4-D has been isolated from pristine Hawaiian volcanic soil. It has been found to contain a *tfdA* gene (encoding the enzyme for the first step of 2,4-D mineralization) that is transmissible to *Ralstonia eutropha* JMP228 carrying a plasmid with a mutant *tfdA* gene (Kamagata et al., 1997). In an analysis of a bacterial community decomposing polyethylene glycol, *V. paradoxus* has been identified as the most active decomposer (Sedina and Ivanov, 1991). *V. paradoxus* is able to degrade poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), and polypropiolactone plastics *in vitro* (Mergaert and Swings, 1996; Kobayashi et al., 1999). *V. paradoxus* is among the most frequently isolated species of polyhydroxyalkanoate-degrading microorganisms from PHB and PHBV plastics buried in soils and sludge, and is sporadically isolated from PHB and PHBV plastics buried in household compost or immersed in fresh water (Mergaert and Swings, 1996). An extracellular PHB depolymerase from *V. paradoxus* S2 has been isolated and characterized (Shiraki et al., 1995; Kobayashi

et al., 1999). *Variovorax* strains from river water have been reported to degrade the aliphatic polycarbonates polyhexamethylene carbonate and polytetramethylene carbonate (Suyama et al., 1998a). *Variovorax* also has been detected in a mixed culture capable of degrading metal–EDTA complexes, isolated from water from the river Mersey (UK) mixed with sludge from an industrial effluent treatment plant (Thomas et al., 1998). In a study of the influence of different chemical treatments on the transport of bacteria through porous media, *V. paradoxus* has been used as a model organism. Retention of bacteria is reduced by treatments making cells more hydrophobic and less electrostatically charged. Such effects may be important in improving the penetration of bacteria used in bioremediation programs of subsurface pollutants (Gross and Logan, 1995). *V. paradoxus* has been found to be a major bacterial group in the rhizosphere of chicory (*Cichorium intybus* L. var. *foliosum* Hegi), mainly early in the growing season (Van Outryve et al., 1988).

ENRICHMENT AND ISOLATION PROCEDURES

Facultatively chemolithotrophic, hydrogen-oxidizing strains of *V. paradoxus* can be isolated from soil, mud, and water by enrichment in the liquid basal mineral medium[1] described by Palleroni and Doudoroff (1972). After inoculation, cultures are incubated at 30°C under an atmosphere of $H_2/O_2/CO_2/N_2$ (50:4–20:5:25–41) (Davis et al., 1970). Heterotrophic strains can be isolated from soil by enrichment with pantothenate (Davis et al., 1970) or poly-3-hydroxybutyrate (Delafield et al., 1965). Other strains have been isolated from soil and water samples using various complex chemicals for enrichment (Ka and Tiedje, 1994; Kamagata et al., 1997; Suyama et al., 1998a; Thomas et al., 1998).

MAINTENANCE PROCEDURES

For heterotrophic growth, convenient media, such as nutrient agar, can be used. Biotype I strains can be maintained under autotrophic or heterotrophic conditions. No reports are available regarding the preservation of autotrophic properties after prolonged periods of heterotrophic growth. However, Aragno and Schlegel (1992) have reported autotrophic cultures to remain viable at 4°C for longer periods (up to 6 months) than do heterotrophic ones.

Variovorax strains can be lyophilized in skim milk in the presence of a suitable cryoprotectant, such as 5% glutamate, 5% *meso*-inositol, or 10% honey (Aragno and Schlegel, 1992). For the preservation of autotrophic properties, the use of autotrophically grown cultures for lyophilization is preferred. Cells can also be stored at −80°C in nutrient broth (or another suitable medium) plus 10% glycerol.

DIFFERENTIATION OF THE GENUS *VARIOVORAX* FROM OTHER GENERA

See Table BXII.β.42 for the family *Comamonadaceae* for features differentiating *Variovorax* from the other genera of this family.

TAXONOMIC COMMENTS

In their phenotypic study of 65 hydrogen bacteria and related nonautotrophic bacteria, Davis et al. (1969) described a group of yellow pigmented, peritrichously flagellated organisms comprising both facultatively autotrophic and nonautotrophic

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1. Basal mineral medium contains: Na-K phosphate buffer (pH 6.8) 0.033 M; NH_4Cl, 0.1%; $MgSO_4·7H_2O$, 0.05%; ferric ammonium citrate, 0.005%; $CaCl_2$, 0.0005% (Palleroni and Doudoroff, 1972).

strains. The authors proposed that the genus *Hydrogenomonas*, which until then had been used to group all Gram-negative facultatively autotrophic, hydrogen-oxidizing bacteria, be abandoned because its type species, *Hydrogenomonas pantropha*, was considered a *nomen dubium*, of which no strains were available or could be isolated. Most of the polarly flagellated *Hydrogenomonas* species could be accommodated in the genus *Pseudomonas*, but the choice was less evident for the peritrichously flagellated species because of the lack of a broadly defined genus with a good type species. By elimination, the only acceptable existing peritrichously flagellated genus with a well defined type species was *Alcaligenes*. Thus, *Alcaligenes paradoxus* was created to comprise the yellow-pigmented, peritrichously flagellated strains, even though these strains did not have a true peritrichous type of flagellation, but rather an oligotrichous flagellation with 1–3 laterally inserted flagella (Aragno et al., 1977). Within *A. paradoxus*, two biotypes were proposed to accommodate the facultatively autotrophic and heterotrophic strains, respectively (Davis et al., 1969). Based on immunological comparisons of glutamine synthetase (Baumann and Baumann, 1978) and DNA–rRNA hybridizations (De Vos and De Ley, 1983), it became clear that *A. paradoxus* was more closely related to the *Pseudomonas acidovorans* group of species than to *Alcaligenes faecalis* and *Alcaligenes eutrophus*. More extensive DNA–rRNA hybridizations and further polyphasic characterization of the acidovorans group finally led to a proposal to transfer *Alcaligenes paradoxus* to a new genus *Variovorax* as *Variovorax paradoxus* (Willems et al., 1991a).

Variovorax is a member of the so-called "acidovorans rRNA complex" (Kersters and De Ley, 1984b), now the family *Comamonadaceae* (Willems et al., 1991a), in the *Betaproteobacteria*. Within this group, it is equidistantly related to each of the other taxa by DNA–rRNA hybridizations. No significant DNA binding is detected between *Variovorax* and representative strains of the other yellow pigmented genera *Hydrogenophaga* and *Xylophilus* (Willems et al., 1991a). In a recent phylogenetic analysis of the 16S rRNA gene sequences of members of the family *Comamonadaceae*, *V. paradoxus* has been found to group with *Xylophilus ampelinus*, a yellow pigmented pathogen of grapevine. The level of 16S rDNA similarity of 97.9% suggests that both species may belong to the same genus (Wen et al., 1999). Until further evidence of genotypic similarities is provided, the phenotypic differences between the slow-growing plant pathogen *Xylophilus* and the more versatile hydrogen-oxidizing *Variovorax* justify maintaining both as separate genera.

FURTHER READING

Aragno, M. and H.G. Schlegel. 1992. The mesophilic hydrogen-oxidizing (Knallgas) bacteria. *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications., 2nd Ed., Vol. 1, Springer-Verlag, New York. pp. 344–384.

List of species of the genus Variovorax

1. **Variovorax paradoxus** (Davis 1969) Willems, De Ley, Gillis and Kersters 1991a, 447[VP] (*Alcaligenes paradoxus* Davis *in* Davis, Doudoroff, Stanier and Mandel 1969, 387.)
   pa.ra.dox′us. Gr. prep. *para* amis, contrary to; Gr. n. *doxus* an opinion; M.L. n. *paradoxus* contrary to expectation, in reference to the chemolithotrophic and/or organotrophic metabolism of the organism.

   The morphological and cellular characteristics are as described for the genus. Additional descriptive information is presented in Table BXII.β.60, which is based on data from Davis et al. (1969, 1970), Kersters and De Ley (1984b), and Aragno and Schlegel (1992).

   Biotype I comprises facultatively autotrophic strains that reduce nitrate to nitrite in organic media. Some strains isolated under low partial pressures of oxygen have been reported to show "oxygen sensitivity" and initially do not grow autotrophically with 20% O_2. These strains can produce mutants indistinguishable with respect to oxygen sensitivity from other strains initially isolated with 20% or 30% O_2 (Davis et al., 1969). Wilde and Schlegel (1982) have reported strains to be tolerant to 80% O_2 when grown autotrophically and 100% O_2 when grown heterotrophically. Autotrophic cultures produce a characteristic unpleasant odor similar to that of soapy water in a laundry (Davis et al., 1969). Typical biotype I strains are the type strain of the species, as well as strains ATCC 17712, ATCC 17715, ATCC 17716, ATCC 17722, and ATCC 17723.

   Biotype II strains are nonautotrophic and rarely reduce nitrate to nitrite. A typical biotype II strain is strain ATCC 17549; other strains are ATCC 17716, ATCC 17719, and ATCC 11720 (Davis et al., 1969). More strains are available in several culture collections, but it is not always known which biotype they represent.

   Isolated by enrichment from soil, mud and water.

   The mol% G + C of the DNA is: 66.8–69.4 (T_m).

   Type strain: ATCC 17713, DSM 30034, IFO 15149, LMG 1797.

Genus Incertae Sedis XI. **Aquabacterium** *Kalmbach, Manz, Wecke and Szewzyk 1999, 775[VP]*

WERNER MANZ, SIBYLLE KALMBACH AND ULRICH SZEWZYK

A.qua.bac′te.ri.um. L. n. *aqua* water; Gr. n. *bakterion* rod; *Aquabacterium* a rod-shaped bacterium isolated from drinking water biofilms.

Rod-shaped cells, 0.5×1–4 µm. **Motile** by means of **monotrichous polar flagella**. Gram negative. **Polyalkanoate** and **polyphosphate** inclusion bodies have been frequently observed as storage materials. **Extracellular polymeric substances are formed,**

even under oligotrophic conditions. **Oxidase positive. Catalase negative. Microaerophilic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Nitrate can be used as an alternate electron acceptor for anaerobic respiration, but nitrite, chlorate, sulfate, and iron (III) are not used as electron acceptors. **Growth by fermentation does not occur. Manganese is not oxidized. Carbohydrates are not used.** Tweens 20, 40, 60, and 80, acetate, butyrate, valerate, caproate, caprylate, succinate, adipate, pimelate, azelate, sebacate, fumarate, β-hydroxybutyrate, malate, and butanol can be used as carbon sources. Starch, esculin, gelatin, and DNA are not hydrolyzed. NaCl levels are tolerated up to 1.8%. Optimal pH, ~7; pH growth range, 5.5–10, depending on the species. Optimal temperature, ~20°C; temperature range, 6–36°C. Habitat: drinking water biofilm originating from various raw water sources (groundwater, surface water, artificially recharged groundwater) used for drinking water production.

The mol% G + C of the DNA is: 65–66.

Type species: **Aquabacterium commune** Kalmbach, Manz, Wecke and Szewzyk 1999, 776.

FURTHER DESCRIPTIVE INFORMATION

Aquabacterium is a slow-growing organism forming colonies of 1.5–3 mm in diameter on solid media after 10 d at 20°C. Colonies on modified R2A agar[1] are flat, transparent, white to cream-white, with a smooth margin and transparent edges. Cells are

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[1] Modified R2A agar (Kalmbach et al., 1999) has the following composition (g/l of distilled water): yeast extract, 0.5; Difco protease peptone no. 3, 0.5; Casamino acids, 0.5; glucose, 0.5; Tween 80, 0.1% (v/v); sodium pyruvate, 0.3; K_2HPO_4, 0.3; $MgSO_4 \cdot 7H_2O$, 0.05; agar, 15. Adjust the medium at final pH 7.2 with crystalline K_2HPO_4 or KH_2PO_4 before adding agar, and sterilize for 15 min at 121°C.

notable for the presence of both polyphosphate and polyalkanoate inclusion bodies (Figs. BXII.β.45 and BXII.β.46).

In a survey of drinking water biofilms grown on different raw water sources in Europe for the occurrence of *Aquabacterium, in situ* probing with fluorescently labeled, rRNA-targeted oligonucleotide probes have shown *Aquabacterium commune* to be a widespread bacterial species (Kalmbach et al., 2000).

ENRICHMENT AND ISOLATION PROCEDURES

For the enrichment and isolation of *Aquabacterium*, bacteria have been detached from young drinking water biofilms that had been grown on glass and polyethylene slides in a modified Robbins device installed in a house installation system at the Technical University of Berlin (Kalmbach et al., 1997). The temperature in the Berlin drinking water varies from 9.4°C to 15.6°C and the pH values ranges from 7.2 to 7.7. Detached bacteria are pooled in a total volume of 2 ml sterile drinking water and vigorously mixed with a vortex mixer. Pure cultures are obtained by plating serial dilutions of the bacterial suspension on R2A agar (Reasoner and Geldreich, 1985) with subsequent incubation at 20°C for 10 d in the dark. Liquid cultures are agitated constantly at 100 rpm at 20°C. For routine cultivation of strains, R2A medium is modified by replacing starch with 0.1% (v/v) Tween 80.

MAINTENANCE PROCEDURES

Aquabacterium can be cultivated on either R2A agar or broth (Reasoner and Geldreich, 1985) that is modified by replacing starch with 0.1% (v/v) Tween 80 (Sigma). Strains grow well in liquid cultures under constant agitation at 100 rpm and 20°C. Storage is possible in the frozen state in culture medium containing 50% (v/v) glycerol. For long-term preservation of *Aquabacterium*, freeze-drying is recommended.
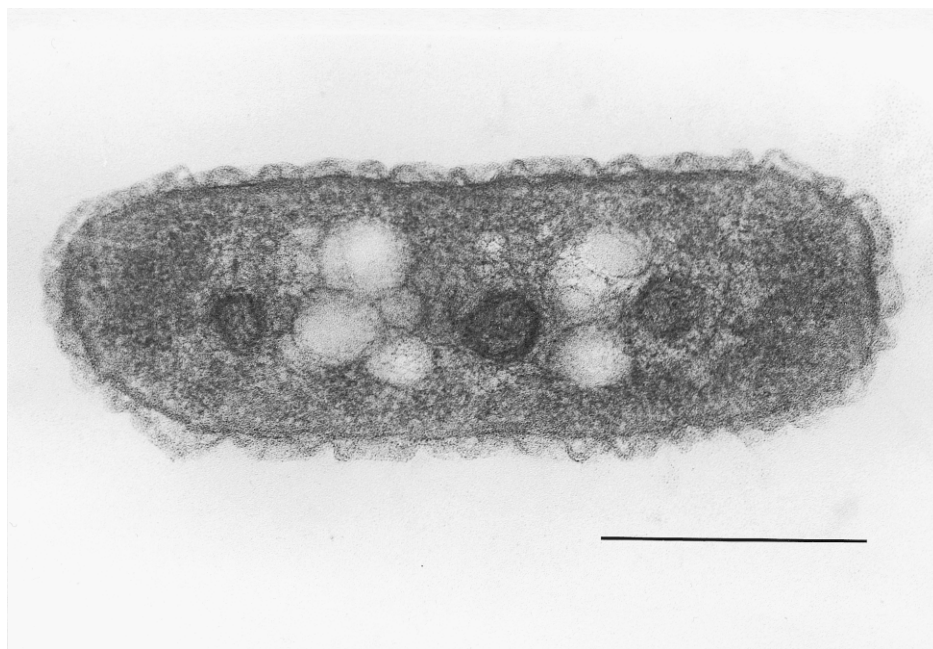

FIGURE BXII.β.45. Transmission electron micrograph of longitudinal thin section of an *A. commune* cell grown in modified R2A liquid medium, showing the typical cell morphology with polyphosphate (black) and polyalkanoate (white) inclusion bodies. Bar = 0.5 μm.
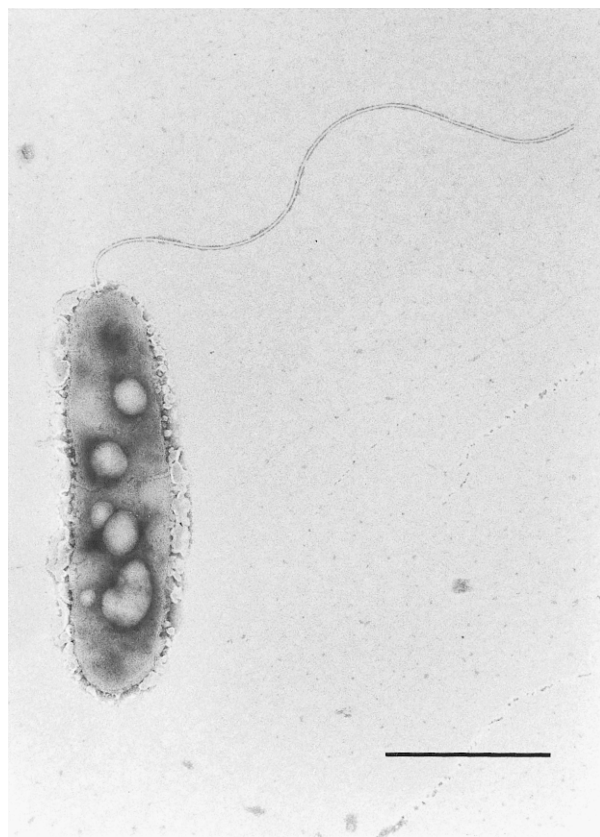
FIGURE BXII.β.46. Thin-section transmission electron micrograph of a negatively stained cell of *A. citratiphilum* showing the polar flagellum, polyphosphate (black), and large polyalkanoate inclusion bodies (white). Flagellation is similar for *A. parvum* and *A. commune*. Bar = 1.0 μm. (Reprinted with permission from S. Kalmbach et al., International Journal of Bacteriology, *49:* 769–777, 1999, ©International Union of Microbiological Societies.)
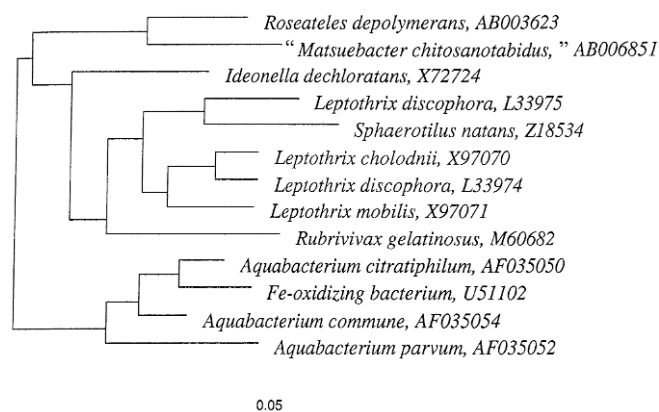

FIGURE BXII.β.47. Phylogenetic tree reflecting the affiliation of the three species of the genus *Aquabacterium* among the next closely related genera within the *Comamonadaceae*. The phylogenetic tree was reconstructed on the basis of full-length 16S rDNA sequences by the maximum parsimony algorithm, using the ARB software package (Strunk and Ludwig, 1998). Bar = 5% sequence divergence.

sheaths. *Leptothrix* can be further differentiated from *Aquabacterium* by its ability to oxidize manganese.

TAXONOMIC COMMENTS

From a phylogenetic perspective, *Aquabacterium* is placed within the class *Betaproteobacteria*, the order *Burkholderiales*, and the family *Comamonadaceae*. At present, a total of 16 genera are included in this family. The genera most closely related to *Aquabacterium* are shown in Fig. BXII.β.47.

Spectrophotometrically determined reassociation rates of the genomic DNA (De Ley et al., 1970a) among the *Aquabacterium* species show the following levels of DNA–DNA relatedness: 44.9% between *A. citratiphilum* and *A. commune*, 45.4% between *A. citratiphilum* and *A. parvum*, and 51.3% between *A. parvum* and *A. commune*. 16S rDNA sequence similarity values are 98.2% between *A. citratiphilum* and *A. commune*, 97.2% between *A. parvum* and *A. commune*, and 96.5% between *A. citratiphilum* and *A. parvum*.

The genus *Aquabacterium* is characterized by an oligonucleotide signature (sequence 5′-CUUGCUGUUCAGUAACGAAGC-3′) within the 16S rRNA located at position 841 according to the *E. coli* numbering (Brosius et al., 1981).

DIFFERENTIATION OF THE GENUS *AQUABACTERIUM* FROM OTHER GENERA

The genus *Aquabacterium* can be differentiated from closely related genera within the family *Comamonadaceae*—particularly the genera *Ideonella* and *Leptothrix*—by its inability to metabolize carbohydrates. In addition, in contrast to *Ideonella dechloratans*, *Aquabacterium* shows no catalase activity and does not reduce chlorate. Members of the genus *Leptothrix* are morphologically characterized by sheath formation, whereas *Aquabacterium* does not form

DIFFERENTIATION OF THE SPECIES OF THE GENUS *AQUABACTERIUM*

The differential characteristics of *A. citratiphilum*, *A. parvum*, and *A. commune* are summarized in Table BXII.β.61. Other characteristics of the species are listed in Table BXII.β.62.

List of species of the genus Aquabacterium

1. **Aquabacterium commune** Kalmbach, Manz, Wecke and Szewzyk 1999, 776^VP

   com′mu.ne. L. adj. *communis, -e* common, referring to the predominance of the species in drinking water biofilms of the Berlin distribution system.

   The characteristics are as given for the genus and as listed in Tables BXII.β.61 and BXII.β.62. Colonies are flat, transparent, have a smooth margin, and are 1.5–2 mm in diameter after 10 d at 20°C on modified R2A agar. *A. commune* is the only species of *Aquabacterium* that utilizes ben-

TABLE BXII.β.61. Differential features of the species of the genus *Aquabacterium*[a]

Property	A. commune	A. citratiphilum	A. parvum
Dimensions (μm)	0.5–2-4	0.5–2-4	0.5–1-2
Temperature range, °C	6–34	10–36	14–34
Tolerated NaCl concentration, %	0–0.4	0–1.8	0–0.8
Urea hydrolysis	−	+	+
Casein hydrolysis	+	−	−
pH range for growth	6.5-9.5	5.5-10.0	6.5-10.0
Carbon and energy sources:			
Bromosuccinate	+	+	−
Propionate	+	+	−
Pyruvate	+	+	−
Benzoate	+	−	−
Casamino acids	+	−	−
Glutamate	+	−	−
Citrate	−	+	−
Glycerol	−	+	−
γ-Hydroxybutyrate	−	+	−
Lactate	−	+	−

[a]Adapted from Kalmbach et al. (1999).

zoate, Casamino acids, and glutamate. Among *Aquabacterium* species, *A. commune* shows the lowest temperature growth limit.

The mol% G + C of the DNA is: 66 (T_m).

Type strain: B8, ATCC BAA-209, CIP 106984, DSM 11901.

GenBank accession number (16S rRNA): AF035054.

2. **Aquabacterium citratiphilum** Kalmbach, Manz, Wecke and Szewzyk 1999, 776[VP]

ci.tra.ti′ phi.lum. L. n. *citrus* lemon tree; L. n. *acidum* acid; L. neut. adj. *acidum citri* citric acid; Gr. adj. *philos* loving; M.L. neut. adj. *citratiphilum* citrate-loving, referring to the preferred utilization of citrate as a carbon and energy source.

The characteristics are as given for the genus and as listed in Tables BXII.β.61 and BXII.β.62. Colonies are flat, cream-white, have a smooth margin, and are 2–3 mm in diameter after 10 d at 20°C on modified R2A agar. The pH range for growth is 5.5–10.0. Differs from other *Aquabacterium* species by its preferred growth on citrate and its utilization of lactate, γ-hydroxybutyrate, and glycerol.

The mol% G + C of the DNA is: 66 (T_m).

Type strain: B4, ATCC BAA-207, CIP 106985, DSM 11900.

GenBank accession number (16S rRNA): AF035050.

3. **Aquabacterium parvum** Kalmbach, Manz, Wecke and Szewzyk 1999, 776[VP]

TABLE BXII.β.62. Other characteristics of the species of the genus *Aquabacterium*[a,b]

Characteristic	A. commune	A. citratiphilum	A. parvum
Rod-shaped cells	+	+	+
Motile by a single polar flagellum	+	+	+
Oxidase test	+	+	+
Catalase test	−	−	−
Reduction of:			
NO_3^-	+	+	+
NO_2^-, Fe^{3+}, SO_4^{2-}, ClO_3^-	−	−	−
Hydrolysis of esculin, DNA, starch, and gelatin	−	−	−
Carbon and energy sources:[c]			
Acetate	+	+	+
Adipate	+	+	+
Azelate	+	+	+
Butanol	+	+	+
Butyrate	+	+	+
Caproate	+	+	+
Caprylate	+	+	+
Fumarate	+	+	+
β-Hydroxybutyrate	+	+	+
Malate	+	+	+
Succinate	+	+	+
Pimelate	+	+	+
Sebacate	+	+	+
Tweens 20, 40, 60, and 80	+	+	+
Valerate	+	+	+
Habitat: drinking water biofilm	+	+	+

[a]Symbols: see standard definitions.

[b]Adapted from Kalmbach et al. (1999).

[c]Substrates which are not utilized by aquabacteria include: *N*-acetylglucosamine, L-arabinose, ascorbate, caprate, ethanol, formate, D-fructose, D-galactose, galacturonate, gluconate, D-glucose, glutarate, glyoxylate, D-lactose, malonate, D-maltose, D-mannose, D-mannitol, D-melibiose, methanol, oxalate, phthalate, L-rhamnose, D-ribose, D-ribulose, sucrose, tartrate, D-trehalose, and D-xylose.

par′ vum. L. adj. *parvus* small.

The characteristics are as given for the genus and as listed in Tables BXII.β.61 and BXII.β.62. Colonies are flat with a smooth margin, white in the center with transparent edges, and 1.5–2 mm in diameter after 10 d at 20°C on modified R2A agar.

The mol% G + C of the DNA is: 65 (T_m).

Type strain: B6, ATCC BAA-208, CIP 106983, DSM 11968.

GenBank accession number (16S rRNA): AF035052.

Genus Incertae Sedis XII. **Ideonella** *Malmqvist, Welander, Moore, Ternström, Molin, Stenström 1994b, 595[VP] (Effective publication: Malmqvist, Welander, Moore, Ternström, Molin, Stenström 1994a, 63)*

ÅSA MALMQVIST, EDWARD R.B. MOORE AND ANDERS TERNSTRÖM

I.de.o.nel′ la. M.L. fem. n. derived from *Ideon* the research center where the bacterium was isolated and described; M.L. dim. ending *ella*.

Cells are straight or slightly curved rods, 0.7–1.0 × 2.5–5.0 μm, occurring singly, in pairs or in short filaments of four to five cells. When occurring in pairs or filaments, individual cells have a fusiform appearance with pointed ends. **Motile by two or more polar or subpolar flagella.** Gram negative. Prosthecae, sheaths, or endospores are not produced. No resting stages are known. Bacteriochlorophyll, carotenoids, or other pigments are not produced. **Aerobic**, having a strictly respiratory metabolism with ox-

ygen as the terminal electron acceptor; however, **chlorate can serve as an alternate terminal electron acceptor under anaerobic conditions**. **Oxidase positive. Weakly catalase positive;** catalase is produced only in minute amounts and the conventional agar colony test may be interpreted as negative. Chemoorganotrophic, utilizing organic acids, amino acids, and carbohydrates as carbon sources. Mesophilic, growing in the temperature range of 12–42°C. Belongs to the class *Betaproteobacteria*.

The mol% G + C of the DNA is: 68.1.

Type species: **Ideonella dechloratans** Malmqvist, Welander, Moore, Ternström, Molin, Stenström 1994b, 595 (Effective publication: Malmqvist, Welander, Moore, Ternström, Molin, Stenström 1994a, 63.)

FURTHER DESCRIPTIVE INFORMATION

Growth of *Ideonella dechloratans* occurs in a simple mineral medium containing an appropriate carbon source, but the growth yield in an anaerobic environment, using chlorate as the electron acceptor, is increased by the addition of 50 mg/l beef extract.

Chlorate is reduced to chloride in an anaerobic environment. The highly oxidized chlorate ion is an excellent electron acceptor for *I. dechloratans*, being even more efficient, theoretically, than oxygen ($\Delta G° = -132$ kJ/mol and -110 kJ/mol of electrons exchanged, respectively), and chlorate-contaminated wastewater effluents have been detoxified by a bacteria-mediated, anaerobic reduction of chlorate to chloride (Malmqvist et al., 1991). In practice, however, *I. dechloratans* prefers oxygen, which is evidenced by the fact that chlorate is not reduced in aerobic conditions.

Nitrate can serve as an alternative electron acceptor, but this ability is frequently lost after repeated subcultures on chlorate. Nitrate is reduced to nitrite.

ENRICHMENT AND ISOLATION PROCEDURES

Ideonella dechloratans was originally enriched anaerobically from a chemostat of chlorate-containing sewage at 37°C, using a defined medium of 10 mM $NaClO_3$, 25 mM acetic acid, 3 mM NH_3, 0.25 mM KH_2PO_4, 0.1 mM $MgSO_4$, 0.1 mM $Ca(OH)_2$, 0.05 mM $FeSO_4·7H_2O$, 0.05 mM $MnSO_4·H_2O$, 5 µM $NiCl·6H_2O$, 5 µM $CoCl_2·6H_2O$, 5 µM $ZnSO_4·7H_2O$, 0.1 µM H_3BO_3, 0.1 µM Na_2SeO_4, 0.1 µM Na_2WO_4, and 0.1 µM Na_2MoO_4. The pH was 7.0. Samples were taken from the reactor and streaked onto the same medium supplemented with 1.5% agar and incubated anaerobically in a GasPak jar (BBL), at 37°C. After 4 d, nonpigmented colonies were observed.

DIFFERENTIATION OF THE GENUS *IDEONELLA* FROM OTHER GENERA

Ideonella is recognized to be related genotypically (i.e., as determined by 16S rRNA gene sequence analysis) to the genera *Aquabacterium* (Kalmbach et al., 1999), *Leptothrix* (Siering and Ghiorse, 1996), and *Roseateles* (Suyama et al., 1999), within the *Betaproteobacteria*. Phenotypically, all of the species of the genera composing the *Ideonella–Aquabacterium–Leptothrix–Roseateles* 16S rDNA phylogenetic lineage produce Gram-negative, motile, rod-shaped cells. They are all chemoorganotrophic, collectively being able to utilize a range of organic acids, amino acids, and carbohydrates. They are all aerobic, possessing a strictly respiratory metabolism. *Ideonella dechloratans* may be distinguished from the species of *Aquabacterium*, *Leptothrix*, and *Roseateles* by its unique ability to reduce chlorate and by the production of catalase. It is differentiated from *Aquabacterium* species in its ability to metabolize

carbohydrates and its ability to grow at temperatures as high as 42°C. It is differentiated from *Leptothrix* species by the absence of sheaths, by possessing more than one polar or subpolar flagellum, and by failing to oxidize Mn^{2+}. It is differentiated from *Roseateles* species by failing to produce bacteriochlorophyll or carotenoids, but is able to reduce nitrate and grow anaerobically.

TAXONOMIC COMMENTS

Ideonella dechloratans was recognized initially as a typical "pseudomonad", i.e., a Gram-negative, respiring, cytochrome *c* oxidase positive, motile rod, capable of utilizing a wide spectrum of organic molecules. At that time, however, the genus *Pseudomonas* was being acknowledged as comprising a range of phylogenetically diverse species, which would be ultimately reclassified in different genera. Analysis of the 16S rDNA gene sequence and estimation of the phylogenetic position (Malmqvist et al., 1994a) demonstrated that *Ideonella* belonged within the β-subclass (Woese et al., 1984c) of the *Proteobacteria* (Stackebrandt et al., 1988b) and was most closely related to species of the *Comamonadaceae* or "acidovorans rRNA complex" (Willems et al., 1991a). However, the analysis shown in Fig. BXII.β.48 indicates that *I. dechloratans* belongs within a phylogenetic lineage related to, yet
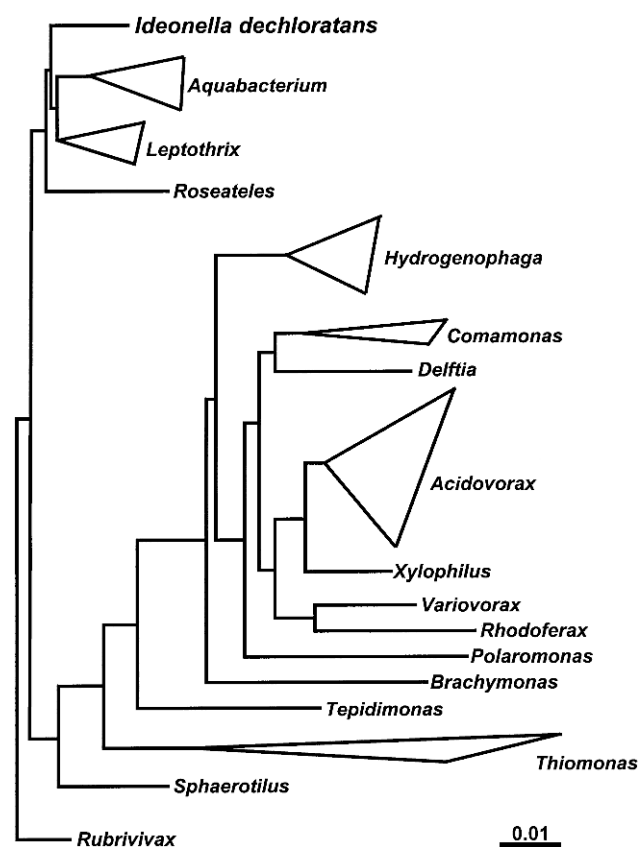
FIGURE BXII.β.48. The inferred phylogenetic position of *Ideonella dechloratans* among other genera of the *Betaproteobacteria*. Evolutionary distances were derived from pair-wise dissimilarities of 16S rRNA gene sequences. The unrooted dendrogram was generated using the neighbor-joining algorithm. The sequences used were obtained from the EMBL Nucleotide Sequence Database (Stoesser et al., 2001) and included those for all validly published species of each genus depicted in the dendrogram. The cluster length for each genus is depicted as the estimated length of the longest branch within each cluster.

distinct from, the *Comamonadaceae* and more closely related to species of the genera *Aquabacterium*, *Leptothrix*, and *Roseateles*.

The species of the genera composing the *Ideonella–Aquabacterium–Leptothrix–Roseateles* 16S rDNA phylogenetic lineage possess DNA composition values ranging from 65 to 71 mol% G + C (*Ideonella*, 68%; *Aquabacterium*, 65–68%; *Leptothrix*, 69–71%; *Roseatels*, 66%). However, *I. dechloratans* is distinguished from the species of *Aquabacterium*, *Leptothrix*, and *Roseateles*, possessing a monophyletic lineage with 16S rDNA gene sequence differences of 3–4%.

It is not clear whether the ability to utilize chlorate as an energy-yielding terminal electron acceptor is limited to a specialized group of bacteria or whether it is distributed throughout different bacterial taxa, as is the ability to grow with nitrate as terminal electron acceptor. Observations of enriched chlorate-reducing bacteria with cell morphologies different from that of

I. dechloratans (Malmqvist et al., 1991) suggest that other chlorate reducers exist, although it is not possible to draw conclusions as to their relatedness to *I. dechloratans*. Other studies have identified N$_2$-fixing bacteria associated with rice, which are closely related to *I. dechloratans*, as determined by 16S rDNA sequence similarities greater than 99% (unpublished, sequence data deposited in the EMBL nucleotide sequence database). The ability to fix N$_2$ has not been determined for *I. dechloratans*. Yet other studies have detected 16S rDNA sequence types (unpublished, sequence data deposited in the EMBL nucleotide sequence database) similar to that of *I. dechloratans* in samples of an industrial, nitrifying/denitrifying activated sludge and in petroleum-contaminated groundwater. Such analyses suggest that other bacteria related to *I. dechloratans* exist within a range of different ecosystems and may or may not have the capability of utilizing chlorate as a terminal electron acceptor.

List of species of the genus Ideonella

1. **Ideonella dechloratans** Malmqvist, Welander, Moore, Ternström, Molin, Stenström 1994b, 595[VP] (Effective publication: Malmqvist, Welander, Moore, Ternström, Molin, Stenström 1994a, 63.)

   de.chlor.at' ans. L. *de* from; M.L. adj. *chloratans* referring to chlorate; M.L. adj. *dechloratans* derived from chlorate; i.e., chlorate-utilizing bacterium.

   The description of the species is the same as that for the genus, with the following additional information. Cells may contain several inclusions of unknown composition. Colonies are circular, smooth, and nonpigmented. Chlorate is reduced to chloride in an anaerobic environment. Nitrate can serve as an alternative electron acceptor, being reduced to nitrite. Nitrite and sulfate do not serve as electron acceptors. Acetate, alanine, asparagine, butyrate, fructose, glucose, lactate, propionate, pyruvate, and succinate are utilized as sole sources of carbon in mineral medium, both

aerobically and anaerobically, in the latter case with chlorate as the electron acceptor. Adipate and fumarate are also utilized, with chlorate, but not with oxygen. Aminobenzoate, phenol, and phenylalanine are not utilized. No acid is produced from cellobiose, glucose, or maltose in Hugh–Leifson medium. Proteolytic activity is produced against casein but not against egg yolk. Lipolytic activity is evident against egg yolk, Tween 20, Tween 40, Tween 60, and Tween 80, but not against Tween 85.

   Mesophilic, growing in the temperature range of 12–42°C; no growth occurs at 10–46°C. No growth occurs in media with 3% NaCl.

   Isolated from anaerobic enrichments of chlorate-containing sewage in a chemostat reactor.

   The mol% G + C of the DNA is: 68 (HPLC) (Mesbah et al., 1989).

   Type strain: CCUG 30898.

   GenBank accession number (16S rRNA): X72724.

Genus Incertae Sedis XIII. **Leptothrix** *Kützing 1843, 184*[AL]

STEFAN SPRING AND PETER KÄMPFER

Lep' to.thrix. Gr. adj. *leptus* fine, small; Gr. n. *thrix* hair; M.L. fem. n. *Leptothrix* fine hair.

Straight rods, 0.6–1.5 × 2.5–15 μm, occurring in chains within a sheath or free swimming as single cells, pairs, or short chains. Most strains are motile by **one polar flagellum**. One species is characterized by holdfasts[1] and a subpolar tuft of flagella. Poly-β-hydroxybutyrate is stored in globules as reserve material. Gram negative. **Sheaths can become encrusted by the deposition of iron and manganese oxides.**

Chemoorganotrophic. Strictly aerobic, respiratory metabolism. Good growth at low oxygen tensions. The optimal temperature for growth is around 25°C, and the optimal pH is 6.5– 7.5. A variety of sugars and organic acids can be used as carbon and energy sources by most species. The need for additional growth factors, including vitamin B$_{12}$, biotin, thiamine, adenine, and guanine, has been reported for several strains (Rouf and Stokes, 1964; Stokes and Johnson, 1965).

Widely distributed in a variety of freshwater habitats, ranging from unpolluted springs and slowly running water rich in soluble iron and manganese compounds to sediments and sewage.

Type species: **Leptothrix ochracea** (Roth 1797) Kützing 1843, 198 (*Conferva ochracea* Roth 1797.)

FURTHER DESCRIPTIVE INFORMATION

For several species of the genus *Leptothrix*, including *L. discophora*, *L. mobilis*, and *L. cholodnii*, 16S rRNA gene sequences have been determined. In phylogenetic trees, these species cluster together with a group of diverse bacteria including *Sphaerotilus natans*, *Ideonella dechloratans*, *Rubrivivax gelatinosus*, *Alcaligenes latus*, *Roseateles depolymerans*, and *Aquabacterium* species. This branch of bacteria is phylogenetically distinct from the *Comamonadaceae* as defined by Willems et al. (1991a) and is also referred to as the *Rubrivivax* subgroup of the *Betaproteobacteria* (Wen et al., 1999). No 16S rRNA gene sequences are currently available for *Leptothrix ochracea*, the type species of the genus, and *L. lopholea*.

Cells are straight rods, which may be as long as 15 μm. They

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1. Evaginations or blebs formed at one cell pole by which organisms attach to walls of containers, submerged plants, stones, and other surfaces.

occur as filaments within characteristic sheaths or free swimming as single cells or short chains. Enlarged cells and coccoid bodies are formed in older cultures of some strains. A typical trait of several strains is false branching, which develops if a cell attaches to an existing filament and forms a new filament.

Sheaths formed by *Leptothrix* species are tube-like, extracellular structures that resemble those of *Sphaerotilus natans*. The sheath consists of a matrix of heteropolysaccharide and protein fibrils associated with the outer membrane of the Gram-negative wall (Emerson and Ghiorse, 1993b). The heteropolysaccharides contain a mixture of uronic acids and amino sugars and are negatively charged. The sheath proteins contain a high proportion of cysteine residues, which covalently cross-link the fibrils by disulfide bonds, resulting in a stable, mesh-like fabric. Numerous free sulfhydryl and carboxyl groups are distributed throughout the sheath and provide sites for binding of metal cations (Emerson and Ghiorse, 1993a).

During growth in media containing iron and manganese, sheaths become impregnated with metal oxides, which give them a yellow to dark-brown appearance. In this way, cells within the sheaths are protected from inhibitory concentrations of soluble iron or manganese compounds in the environment (Rogers and Anderson, 1976a, b). Further deposition of metal oxides can lead to massive incrustations, thereby increasing the diameter of the bare sheaths several fold (Fig. BXII.β.49). The deposition of iron and manganese oxides by *Leptothrix* species appear to be separate processes, which are catalyzed by proteins. The corresponding enzymes are excreted into the medium or bound to the extracellular sheath (Adams and Ghiorse, 1987; Corstjens et al., 1992). Several studies have shown that *Leptothrix* species do not gain energy from the oxidation of iron or manganese (Van Veen et al., 1978). Nevertheless, it has been observed that the addition of Mn^{2+} to the medium stimulates growth. It has been postulated that this beneficial effect may be mainly due to the oxidation product, MnO_2, which catalytically inactivates H_2O_2, which is ex-

creted in toxic amounts by *Leptothrix* species during growth (Dubinina, 1978a, b).

Isoprenoid quinone Q-8 is the predominant quinone type of *L. discophora* and *L. cholodnii* (Kämpfer, 1995). The fatty acid composition, as determined by gas chromatographic analysis, of strains representing *L. discophora*, *L. cholodnii*, and *L. mobilis* differ only slightly (Spring et al., 1996). All strains contain the fatty acids dodecanoic acid, hexadecanoic acid, *cis*-9-hexadecenoic acid, *cis*-9,11-octadecenoic acid, and 3-hydroxydecanoic acid; this fatty acid profile is typical among members of the *Betaproteobacteria*.

The size and shape of colonies depends largely on the ability of the respective strain to form sheaths (Fig. BXII.β.50). Rough and filamentous colonies (Fig. BXII.β.50a) are characteristic of sheath-forming cells, whereas smooth and regular colonies (Fig. BXII.β.50b) consist mainly of sheathless cells. Due to the oxidation of iron and manganese, a black-brown precipitate is formed within the colonies and occasionally also as a halo around them. Cells tend to aggregate and form flocs in liquid culture.

ENRICHMENT AND ISOLATION PROCEDURES

Several methods for the enrichment of *Leptothrix* species from the environment are based on the tendency of these filamentous organisms to attach to surfaces. Mulder and van Veen (1963) have used continuous-flow devices in order to imitate the natural growth conditions of these organisms. A continuous flow of a dilute nutrient solution that has been enriched with soluble iron and manganese compounds is guided through an apparatus consisting of several vessels arranged one after the other. As in unpolluted, slowly running water, bacteria that are able to attach to solid surfaces have a selective advantage within this device, because they obtain more nutrients than do bacteria moving with the medium. An alternative method that is less laborious has been applied by Spring et al. (1996). Microscope slides are put into a sample of freshwater sediment, and after several weeks,
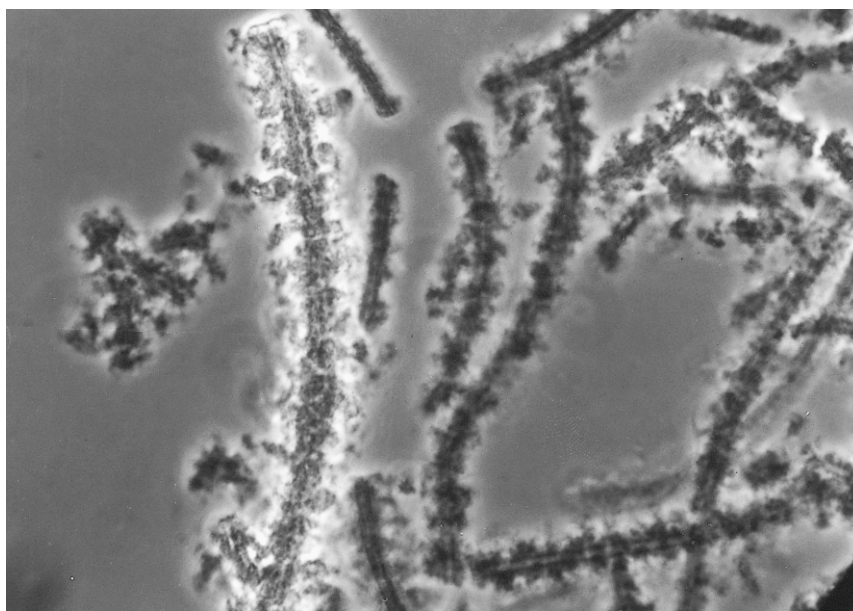

FIGURE BXII.β.49. Unknown strain, presumably belonging to *L. discophora*, grown under laboratory conditions in slowly running iron- and manganese-containing soil extract. Sheaths are covered with Fe(III) and Mn(IV) oxides. Light micrograph ($\times$ 1268).
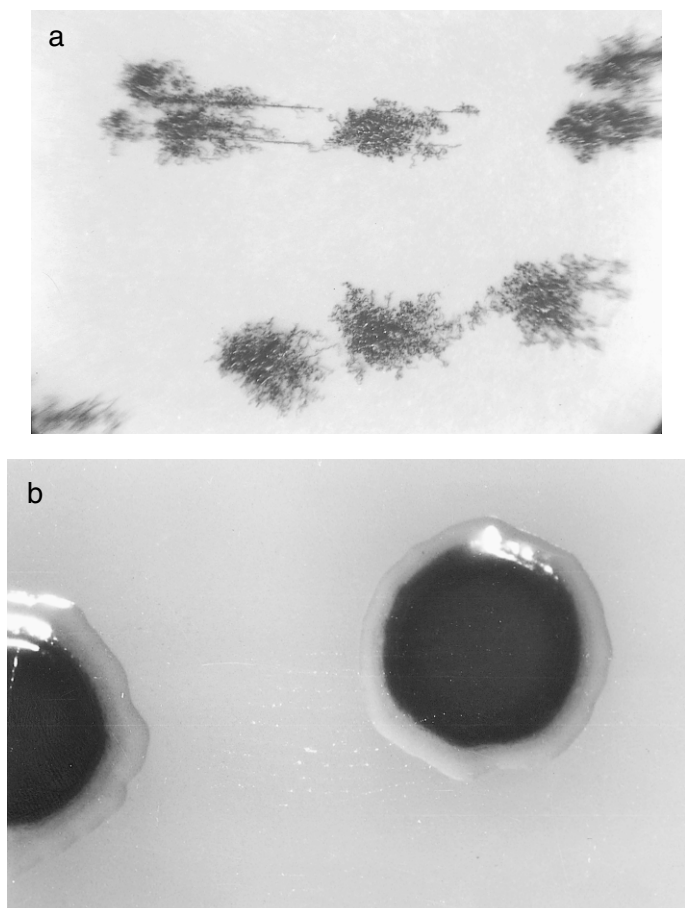
FIGURE BXII.β.50. Examples of various colony morphologies of *Leptothrix* strains grown on Mn(II)-containing agar media. (*a*) Filamentous colonies of an unknown strain presumably belonging to *L. discophora*. Light micrograph (× 18). (*b*) Smooth colonies of *L. mobilis* Feox-1 (DSM 10617). Light micrograph (× 29)

sheaths of filamentous bacteria encrusted with iron and manganese oxides covered parts of the slides. Subsequently, the slides may be removed from the sediment and used for isolation. Another simple enrichment method has been introduced by Rouf and Stokes (1964), who filled glass cylinders with water taken from the environment and added extracted alfalfa straw as a nutrient source, as well as $MnCO_3$ and freshly precipitated $Fe(OH)_3$. After several days, the flocculent growth of sheathed bacteria adhering to the walls of the cylinder indicates the enrichment of *Leptothrix* bacteria.

Isolation of pure cultures may be achieved by streaking material from enrichment cultures on previously dried agar plates containing low levels of nitrogen and carbon sources. The isolation medium used by Rouf and Stokes (1964) has the following composition (per liter tap water): peptone, 5.00 g; ferric ammonium citrate, 0.15 g; $MgSO_4 \cdot 7H_2O$, 0.20 g; $CaCl_2$, 0.05 g; $MnSO_4 \cdot H_2O$, 0.05 g; $FeCl_3 \cdot 6H_2O$, 0.01 g; agar, 12.00 g.

Colonies of *Leptothrix* strains can be easily distinguished from most contaminating bacteria on this medium by their dark-brown color.

Enrichment cultures may not be necessary if natural environments are studied where *Leptothrix* bacteria can be detected by their flocculent growth. Flocculent cell material from these sites may be washed several times with sterile tap water and streaked directly on solid media.

MAINTENANCE PROCEDURES

Stock cultures can be stored on agar slants of the medium of Rouf and Stokes (1964) at 4°C for about three months. Most *Leptothrix* strains do not survive lyophilization. For the long-term preservation of these strains, freezing in liquid nitrogen is recommended.

DIFFERENTIATION OF THE GENUS *LEPTOTHRIX* FROM OTHER GENERA

The genus that is most closely related to *Leptothrix* is *Sphaerotilus*. Traits that distinguish both genera are discussed in the corresponding section of the genus *Sphaerotilus* and listed in Table BXII.β.66 of that chapter.

TAXONOMIC COMMENTS

Some distinguishing characteristics of *Leptothrix* species are not very stable and are regularly lost upon cultivation. The ability to form sheaths has been irreversibly lost by most strains available from public culture collections, with the exception of *Leptothrix cholodnii* LMG 8142. The manganese-oxidizing activity is independent from the formation of sheaths and is more stable in most strains, but loss of this trait has also been reported.

A major problem in the taxonomy of the genus *Leptothrix* is the availability of reference strains. The type species of the genus *Leptothrix*, *L. ochracea*, has not been cultured, and the description is based only on morphological observations. The type strains of *Leptothrix lopholea*, LVMW 124, and *L. cholodnii*, LVMW 99, are not available from public culture collections and have apparently been lost. The strain LMG 7171 may be used as reference strain for *L. cholodnii* until a neotype is designated, but for *L. lopholea*, no other strains are available, preventing detailed taxonomic studies on this species.

Thus, species descriptions of *L. ochracea*, "*L. pseudo-ochracea*", and *L. lopholea* have not been emended and adopted from the first edition of *Bergey's Manual of Systematic Bacteriology* (Mulder, 1989a). Emended or new descriptions of *L. discophora*, *L. cholodnii*, and *L. mobilis* are based on the work of Spring et al. (1996).

FURTHER READING

Spring, S., P. Kämpfer, W. Ludwig and K.-H. Schleifer. 1996. Polyphasic characterization of the genus *Leptothrix*: new descriptions of *Leptothrix mobilis* sp. nov. and *Leptothrix discophora* sp. nov. nom. rev. and emended description of *Leptothrix cholodnii* emend. Syst. Appl. Microbiol. *19*: 634–643.

van Veen, W.L., E.G. Mulder and M.H. Deinema. 1978. The *Sphaerotilus–Leptothrix* group of bacteria. Microbiol. Rev. *42*: 329–356.

List of species of the genus Leptothrix

1. **Leptothrix ochracea** (Roth 1797) Kützing 1843, 198[AL] (*Conferva ochracea* Roth 1797.)
   o.chra' ce.a. Gr. n. *ochra* yellow-ochre; M.L. adj. *ochracea* like ochre.

L. ochracea is probably the most common iron-precipitating ensheathed bacterium all over the world, occurring in slowly running ferrous iron-containing waters poor in readily decomposable organic matter. Its contribution to

the oxidation of Fe^{2+} is uncertain, since (a) the organism normally grows at a pH value of 6–7, at which Fe (II) is readily oxidized nonbiologically, and (b) pure cultures of this bacterium have never been obtained. For the latter reason, its Mn (II)-oxidizing capacity, which probably occurs under natural conditions, has never been confirmed. The pronounced development and activity of *L. ochracea* in iron- and manganese- containing waters give rise to the accumulation and deposition of large masses of ferric oxide and, probably, MnO_2, which are thought to be responsible for the formation of bog ore (see, for instance, Ghiorse and Chapnick, 1983).

Authors who have studied and described *L. ochracea* under natural conditions have been unable to obtain pure cultures (Cholodny, 1926; Charlet and Schwartz, 1954). Those who thought they had isolated *L. ochracea* had, in fact, described one of the other species of this genus (Winogradsky, 1888, 1922; Molisch, 1910; Lieske, 1919; Cataldi, 1939; Pråve, 1957).

The most typical characteristic of *L. ochracea* is the formation of large numbers of almost empty sheaths within a relatively short time. The mechanism of this procedure can be followed under a phase-contrast microscope in a slide culture of the organism in an iron-containing soil extract medium. In this way, the behavior of *L. ochracea* in crude culture can be observed continuously. Chains of cells leave their sheaths at the rate of 1–2 μm/min, continuously producing new hyaline sheaths connected with the old envelope (Fig. BXII.β.51). Impregnation and covering of the sheaths with iron probably take place after the cells have left the envelopes. Aged, golden-brown sheaths are brittle and easily broken into relatively short fragments (Fig. BXII.β.52).

Isolation of *L. ochracea* has not been achieved, either from natural enrichments or in the laboratory from enrichment cultures of slowly running soil extract, so few details of the organism are available. In some instances, an organism resembling *L. ochracea* has been isolated, viz. "*L. pseudo-ochracea*".

The mol% G + C of the DNA is: not available.

Type strain: No culture available.

2. **Leptothrix cholodnii** Mulder and van Veen 1963, 137[AL]

cho.lod′ ni.i. M.L. gen. n. *cholodnii* of Cholodny, named for N. Cholodny, a Russian bacteriologist.

Cells of freshly isolated strains are usually found in long chains inside the sheaths. Single, motile cells may be seen outside the sheaths. In the presence of Mn^{2+}, the cells become covered with granular MnO_2 (Fig. BXII.β.53). At some sheath locations, the MnO_2 deposits may even exceed 10 μm. *L. cholodnii*, in contrast to other *Leptothrix* species, responds to an increased supply of organic nutrients (Table BXII.β.63), resulting in relatively large colonies (up to 5 mm in diameter) on nutrient-rich agar media. On manganese (II)-containing agar, black-brown hairy colonies are formed, particularly when the organism is seeded densely. Most strains display a strong tendency to dissociate spontaneously and to produce smooth, rather than the typical rough, colonies. Such mutant strains are largely sheathless and oxidize manganese slightly or not at all (Mulder and van Veen, 1963; Rouf and Stokes, 1964; Stokes and Powers, 1965).

Growth takes place between 10 and 35°C; the pH range for growth is from pH 6.5 to 8.5. Oxidase positive. The following compounds support growth in GMBN medium (Kämpfer et al., 1995): DL-3-hydroxybutyrate, DL-lactate, L-glutamate, L-leucine, L-proline, phenylpyruvate, and quinate; no growth is obtained with D-fucose, fumarate, or glutarate. Poly-β-hydroxybutyrate granules are formed.

Major fatty acids (>5% each) present in all strains are

FIGURE BXII.β.51. *L. ochracea* in iron- and manganese-containing soil extract. Cells are continuously leaving sheaths and forming new sheaths. Light micrograph ($\times$ 1268).

FIGURE BXII.β.52. Broken empty sheaths of *L. ochracea* in crude culture in slowly flowing iron- and manganese-containing soil extract. Light micrograph (× 1186). (Reproduced with permission from E.G. Mulder and W.L. van Veen, Antonie van Leeuwenhoek Journal of Microbiology and Serology *29:* 121–153, 1963, ©Kluwer Academic Publishers.)

TABLE BXII.β.63. Differential characteristics of *Leptothrix* species[a]

Characteristic	*L. ochracea*	*L. cholodnii*	*L. discophora*	*L. lopholea*	*L. mobilis*	"*L. pseudo-ochracea*"
Cell dimensions:						
Width (µm)	1	0.7–1.5	0.6–0.8	1.0–1.4	0.6–0.8	0.8–1.3
Length (µm)	2–4	2.5–15	2.5–12	3–7	1.5–12	5–12
Monotrichous polar flagella	+	+	+	−	+	+
Polytrichous subpolar flagella	−	−	−	+	−	−
Holdfasts	−	−	−	+	−	−
False branching	−	−	+	+	−	−
Growth at:						
35°C	nd	+	−	nd	+	nd
pH 8.5	nd	+	−	nd	+	nd
Growth on:[b]						
D-Fucose	nd	−	+	nd	−	nd
Fumarate	nd	−	−	nd	+	nd
DL-Lactate	nd	+	−	nd	−	nd

[a]For symbols see standard defininitions; nd, not determined.

[b]Growth was determined in GMBN medium supplemented with the respective carbon source (Kämpfer et al., 1995).

hexadecanoic acid, *cis*-9-hexadecenoic acid, dodecanoic acid, and *cis*-9,11-octadecenoic acid. Tetradecanoic acid is found in small amounts in all strains, whereas other minor fatty acids have not been detected.

Results of DNA–DNA hybridization experiments have indicated that, currently, three different strains belong to this species, viz. LMG 8142, LMG 8143, and LMG 7171, sharing DNA–DNA similarity values well above 70% (Spring et al., 1996). The strain LMG 7171 served as the reference strain in this study, because the original designated type strain has apparently been lost.

In agreement with its nutritional requirements, *L. cholodnii* is found in slowly running iron- and manganese-containing, unpolluted waters or in polluted waters, particularly in activated sludge.

The mol% G + C of the DNA is: 68–70 (T_m).

Type strain: LVMW 99 (not available).

3. **Leptothrix discophora** Spring, Kämpfer, Ludwig and Schleifer 1997, 601[VP] (Effective publication: Spring, Kämpfer, Ludwig and Schleifer 1996, 640.)

dis.co' phor.a. Gr. n. *discos* a disk; Gr. adj. *phoros* bearing; M.L. adj. *discophora* disk-bearing.

Cells are relatively small compared to those of the other *Leptothrix* species described (Table BXII.β.63). They may occur in narrow sheaths or be free swimming; free cells are motile by thin polar flagella at one or both poles. The manganese-oxidizing and ferric oxide-storing capacities of this organism are very pronounced. In enrichment cultures containing nutrient media with Mn (II) salts, the sheaths are heavily but irregularly encrusted with MnO_2, giving rise to sheaths of up to 10 µm thickness. In media with both
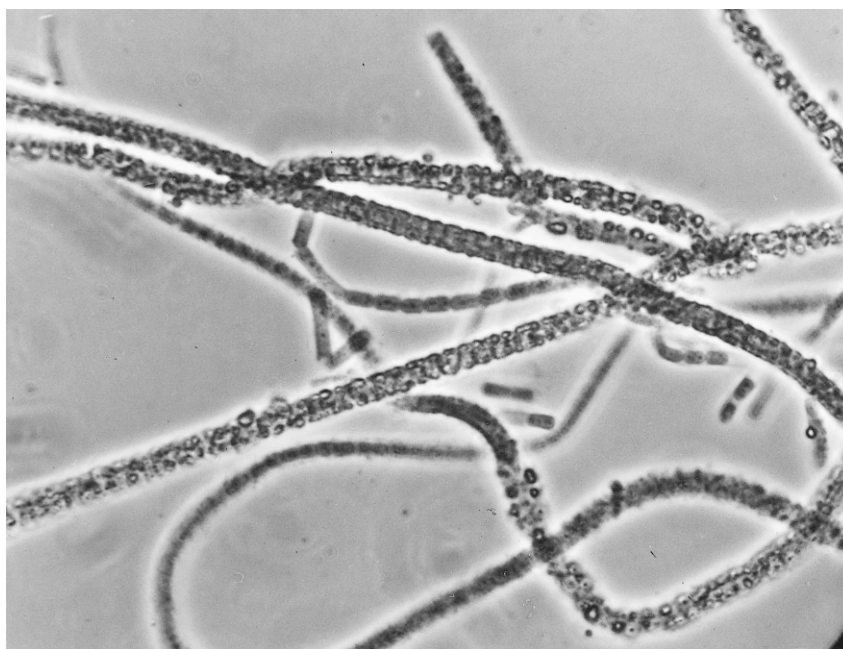
FIGURE BXII.β.53. *L. cholodnii* sheaths encrusted with granulated MnO_2 ($\times$ 1280). (Reproduced with permission from E.G. Mulder and W.L. van Veen, Antonie van Leeuwenhoek Journal of Microbiology and Serology *29:* 121–153, 1963, ©Kluwer Academic Publishers.)

Mn (II) and Fe (II), as in slowly flowing soil extract, the sheaths become covered with a thick, dark brown, fluffy layer of ferric oxide and MnO_2, which may increase the diameter of the trichomes to up to about 20–25 µm (Fig. BXII.β.49). Under these conditions, the sheaths may taper toward the growing tips. Following isolation, the ability to form sheaths is easily lost in this species (Adams and Ghiorse, 1986). False branching is regularly observed, even with sheathless strains. In older cultures, coccoid bodies and cell evaginations are formed.

Colonies on the solid medium of Rouf and Stokes are about 1 mm in diameter, irregular in shape, flat, and dark-brown. Under certain conditions, filamentous colonies may be formed. Increasing the supply of nutrients, such as glucose, peptone, methionine, purine bases, vitamin B_{12}, biotin, and thiamine, only increases growth slightly. Visible aggregates are formed when cells are grown in liquid media.

Growth occurs between 15 and 33°C; the pH range for growth is from pH 6.0 to 8.0. Oxidase positive. D-fucose, L-proline, and protocatechuate support growth in GMBN medium; no growth is obtained with fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-glutamate, L-leucine, phenylpyruvate, and quinate. Poly-β-hydroxybutyrate granules are formed. Major fatty acids (>5%) are hexadecanoic acid, *cis*-9-hexadecenoic acid and dodecanoic acid.

Minor fatty acids are *cis*-9,11-octadecenoic acid, 3-hydroxydecanoic acid and octadecanoic acid.

The normal habitat is slowly running, unpolluted, iron- and manganese-containing water of ditches, rivers, and ponds.

The mol% G + C of the DNA is: 71 (T_m).
Type strain: SS-1, LMG 8141.
GenBank accession number (16S rRNA): L33975.

4. **Leptothrix lopholea** Dorff 1934, 33[AL]
loph.o.le′ a. Gr. n. *lophos* a crest; M.L. dim. fem. adj. *lopholea* somewhat crested or tufted.

L. lopholea resembles *S. natans* to a greater extent than do the other *Leptothrix* species. It produces polytrichous, subpolar flagellation and forms holdfasts and false branches. Strains may also grow in rich media. Cells usually develop short, sheathed filaments radiating from a cluster of holdfasts, giving rise to many tiny flocs when the cells are grown in liquid media (Fig. BXII.β.54). Deposition of iron and manganese oxides is more pronounced on holdfasts than on filaments. On Mn (II)-containing agar media, encrustation of sheaths with MnO_2 is retarded, so that colonies are white at first and later become black-brown. Cell growth responds poorly to an increased supply of organic nutrients. Strains that do show a good response oxidize manganese more slowly.

This species may be isolated from slowly flowing, unpolluted or polluted freshwater and from activated sludge.
The mol% G + C of the DNA is: not available.
Type strain: LVMW 124 (not available).

5. **Leptothrix mobilis** Spring, Kämpfer, Ludwig and Schleifer 1997, 601[VP] (Effective publication: Spring, Kämpfer, Ludwig and Schleifer 1996, 640.)
mo′ bi.lis. L. adj. *mobilis* motile, movable.

Cells are similar in width to those of *L. discophora*, but are usually shorter. They are highly motile by single polar flagella. Sheaths are not formed under laboratory conditions. Colonies on the agar medium of Rouf and Stokes are about 1 mm in diameter, circular, flat, smooth, and dark-brown. Visible aggregates are formed when cells are grown in liquid media.
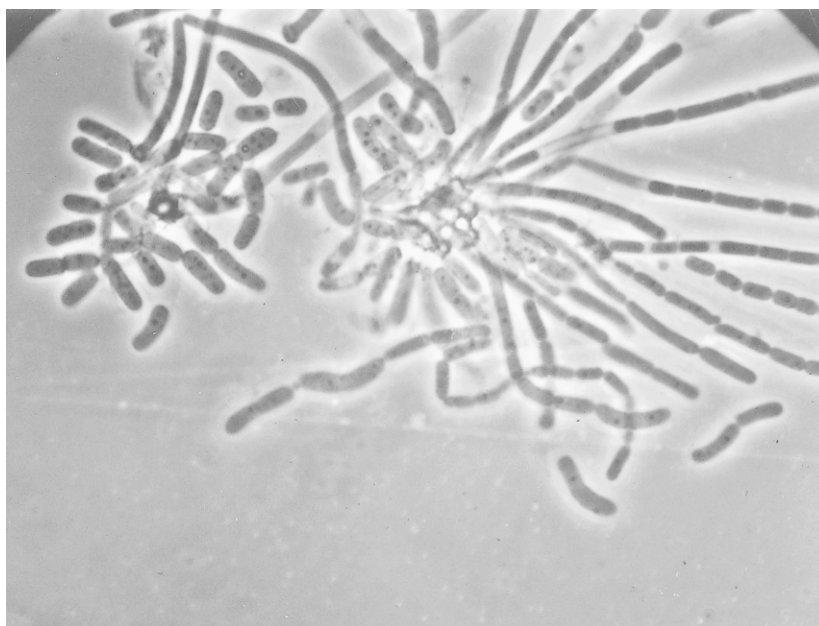
FIGURE BXII.β.54. *L. lopholea* in culture solution. Many trichomes can be seen radiating from common holdfasts. Light micrograph (× 1316). (Reproduced with permission from E.G. Mulder and W.L. van Veen, Antonie van Leeuwenhoek Journal of Microbiology and Serology *29:* 121–153, 1963, ©Kluwer Academic Publishers.)

Growth takes place between 10 and 37°C; the pH range for growth is from pH 6.5 to 8.5. Oxidase positive. Fumarate and glutarate support growth in GMBN medium; no growth is obtained with D-fucose, DL-3-hydroxybutyrate, DL-lactate, L-glutamate, L-leucine, L-proline, phenylpyruvate, protocatechuate, and quinate. Poly-β-hydroxybutyrate granules are formed.

Major fatty acids (>5%) are hexadecanoic acid, *cis*-9-hexadecenoic acid, and *cis*-9,11-octadecenoic acid. Minor fatty acids are 3-hydroxydecanoic acid, dodecanoic acid, octadecanoic acid, tetradecanoic acid, and *cis*-9,10-methyleneoctadecanoic acid.

Isolated from the sediment of a freshwater lake (Chiemsee) in Upper Bavaria, Germany.

The mol% G + C of the DNA is: 68 (T_m).

Type strain: Feox-1, LMG 17066, DSM 10617.

GenBank accession number (16S rRNA): X97071.

6. **"Leptothrix pseudo-ochracea"** Mulder and van Veen 1963, 135.

pseu.do.o.chra' ce.a. Gr. adj. *pseudes* false; M.L. adj. *ochracea* specific epithet; M.L. adj. *pseudo-ochracea* not the true *L. ochracea.*

Cells are more slender than those of the other *Leptothrix* species (Table BXII.β.63), and are very motile by single, thin, polar flagella. Even chains of 6–10 cells may show an undulatory locomotion after leaving their sheaths. This characteristic may account for the relatively large number of empty sheaths in culture, as compared with the number found in most *Leptothrix* species; however *L. ochracea* possesses even more empty sheaths. In slowly flowing ferrous iron-containing soil extract, the sheaths become impregnated with ferric oxide and appear yellow-brown. In this respect, the organism resembles *L. ochracea*. In media with added manganese compounds, the sheaths are covered with small granules of MnO_2. On manganese (II)-containing agar, the black-brown colonies are very filamentous and may exceed a width of 10 mm. On basal agar media containing 0.1% peptone and 0.1% glucose, the organism may grow in concentric rings.

The normal habitat of *"Leptothrix pseudo-ochracea"* is the slowly running, unpolluted, iron- and manganese-containing freshwater of ditches and brooklets. This species may also be found in slightly polluted water.

Deposited strain: none designated.

Genus Incertae Sedis XIV. **Roseateles** Suyama, Shigematsu, Takaichi, Nodasaka, Fujikawa, Hosoya, Tokiwa, Kanagawa and Hanada 1999, 455[VP]

AKIRA HIRAISHI AND JOHANNES F. IMHOFF

Ro.se.a.te' les. L. adj. *roseus* rose-colored, pink; Gr. adj. *ateles* defective, incomplete; M.L. masc. n. *Roseateles* the rose-colored incomplete (photosynthetic bacterium).

Cells are straight slender rods, 0.5–0.6 μm wide, motile by means of polar flagella, reproduce by binary fission. Gram negative. **Belong to the** *Betaproteobacteria.* Do not form any type of internal membranes. **Synthesize bacteriochlorophyll *a* and carotenoids as photosynthetic pigments.**

Strictly aerobic, chemoorganoheterotrophic bacteria. Do not grow and produce photosynthetic pigments under anoxic conditions in the light. Carbon sources and electron donors supporting growth are simple organic compounds, Casamino acids, peptone, skim milk, and yeast extract. Accumulate poly-β-hy-

droxybutyrate. Catalase negative, oxidase positive. **The major respiratory quinone is ubiquinone-8.**

Mesophilic and neutrophilic freshwater bacteria.

The mol% G + C of the DNA is: 66.2–66.3.

Type species: **Roseateles depolymerans** Suyama, Shigematsu, Takaichi, Nodasaka, Fujikawa, Hosoya, Tokiwa, Kanagawa and Hanada 1999, 455.

FURTHER DESCRIPTIVE INFORMATION

The genus *Roseateles* is at this time monotypic, with *R. depolymerans* as the type species (Suyama et al., 1999). Based on 16S rDNA sequence analysis, *R. depolymerans* belongs to the *Betaproteobacteria*, with *Rubrivivax*, *Ideonella*, *Leptothrix*, and *Sphaerotilus* species as phylogenetic neighbors. The 16S rDNA sequence of the phototrophic, purple, nonsulfur bacterium *Rubrivivax gelatinosus* is 96.3% similar to that of *Roseateles depolymerans*.

Colonies on agar media are circular, smooth, and soft. Rough and opaque colonies occur in some cases. The color of colonies is white at the early stage of cultivation but becomes pink in older cultures due to the production of carotenoids. Liquid cultures exhibit no or little pigmentation. The intensity of colony pigmentation depends upon the growth medium. The best medium for pigment production is an agar medium containing 0.02% Casamino acids. Thin-section electron microscopy indicated the absence of any type of internal membranes and the presence of poly-β-hydroxybutyrate granules (cells stained positively with Nile blue A).

Photosynthetic pigments are bacteriochlorophyll *a* esterified with phytol and carotenoids. The major carotenoid of *R. depolymerans* is spirilloxanthin (88–89 mol% of the total content). Biosynthetic precursors of spirilloxanthin, OH-spirilloxanthin, anhydrorhodovibrin, and 3,4-dehydrorhodopin are present as minor components. The molar ratios of total carotenoids/bacteriochlorophyll *a* were 0.58–0.65 (Suyama et al., 1999). Ultrasonically disrupted cells have absorption maxima at 482, 515, 550, 590, 800, and 870 nm. The spectral pattern in the near-infrared region indicates that the cells contain a core light-harvesting complex (B870, LH I), together with the photosynthetic reaction center, but lack a peripheral light-harvesting complex (LH II). Like other aerobic bacteriochlorophyll-containing bacteria, *R. depolymerans* produces BChl *a* only under aerobic growth conditions in darkness.

R. depolymerans is an obligately aerobic chemoorganoheterotrophic bacterium, which is unable to grow under anaerobic conditions, neither in the dark nor in the light. The doubling time exhibited under optimal growth conditions is approx. 2 h. One of the most characteristic features of *Roseateles depolymerans* is the ability to degrade aliphatic polycarbonates, including poly(tetramethylene carbonate), poly(hexamethylene carbonate), and poly(ε-caprolactone), which are known as biodegradable plastics. On polycarbonate agar plates, clear zones are produced around the colonies and the turbidity of opaque polycarbonate suspension in liquid culture is reduced within a few days of incubation. Gel permeation chromatography showed that poly(hexamethylene carbonate) was degraded via the production of diester, di(6-hydroxyhexyl) carbonate (Suyama et al., 1998a). The accumulation of diesters as intermediate products suggests

that lipolytic enzymes may be the key enzymes in this degradation process.

Strains of *R. depolymerans* were isolated together with other polycarbonate-degrading bacteria from Hanamuro River in Ibaraki Prefecture, Japan (Suyama et al., 1998a). Polycarbonate-degrading pigmented strains that are phylogenetically very close to *R. depolymerans* have also been isolated from soil environments (Suyama et al., 1998b). In view of these results, *R. depolymerans* and related bacteria may inhabit a wide variety of freshwater and terrestrial environments.

ENRICHMENT AND ISOLATION PROCEDURES

Surface water of freshwater environments is a possible source for *Roseateles*. For selective enrichment and isolation, the use of mineral medium supplemented with poly(hexamethylene carbonate) is recommended (Suyama et al., 1998a). The polycarbonate liquid medium is inoculated with a suitable water sample and incubated with shaking at 35°C in darkness. Cultures positive for reducing the turbidity of polycarbonate are transferred to agar media of the same composition. After incubation for at least one week, pink colonies with clear zones may appear on the agar medium. Growth media and cultural conditions commonly used for the isolation of freshwater aerobic bacteria can be used for the isolation of *R. depolymerans*.

MAINTENANCE PROCEDURES

Cultures are well-preserved in liquid nitrogen or by lyophilization. Preservation in a mechanical freezer at −80°C is also possible.

DIFFERENTIATION OF THE GENUS *ROSEATELES* FROM OTHER GENERA

Differential characteristics of the genus *Roseateles* and related genera are shown in Table BXII.β.64.

TAXONOMIC COMMENTS

Strains of *Roseateles depolymerans* were first isolated as obligately aerobic chemoorganotrophic bacteria capable of degrading aliphatic polycarbonates (Suyama et al., 1998a). Detailed phenotypic studies showed that these polycarbonate-degrading strains produce bacteriochlorophyll *a* and carotenoids with spirilloxanthin as the main component. However, photosynthetic pigments were formed only under aerobic growth conditions, as found in other obligately aerobic bacteriochlorophyll-containing bacteria. 16S rDNA-based sequence analysis indicated that the polycarbonate-degrading, bacteriochlorophyll-producing strains were closely related to the phototrophic, purple, nonsulfur bacterium *Rubrivivax gelatinosus*. However, major phenotypic differences between the polycarbonate-degrading strains and *Rubrivivax gelatinosus* and other phylogenetically related bacteria led to the proposal of a new genus and species, *Roseateles depolymerans* (Suyama et al., 1999). The genus *Roseateles* belongs to the *Betaproteobacteria* and is the first described genus of aerobic bacteriochlorophyll-containing bacteria that does not fall within the *Alphaproteobacteria*.

ACKNOWLEDGMENTS

List of species of the genus Roseateles

1. **Roseateles depolymerans** Suyama, Shigematsu, Takaichi, Nodasaka, Fujikawa, Hosoya, Tokiwa, Kanagawa and Hanada 1999, 455[VP]

de.po.ly′ me.rans. M.L. v. *depolymerare* depolymerize; M.L. part. adj. *depolymerans* depolymerizing.

Cells are straight slender rods, 0.5–0.6 × 2–5 μm, motile

TABLE BXII.β.64. Differential characteristics of *Roseateles depolymerans* and phylogenetically related bacteria[a]

Characteristic	*Roseateles depolymerans*	*Alcaligenes latus*	*Ideonella dechloratans*	*Leptothrix discophora*	*Rubrivivax gelatinosus*	*Sphaerotilus natans*
Mode of flagellation:						
Lateral		+				
Polar tuft	+		+			+
Single polar				+	+	
Sheath formation	−	−	−	−	−	+
PHB deposition	+	+	nd	+	nd	+
Bacteriochlorophyll *a*	+	−	−	−	+	−
Carotenoid pigment:						
Sprilloxanthin	+				+	
Spheroidene					+	
OH-spheroidene					+	
Anaerobic phototrophy	−	−	−	−	+	−
Autotrophy with H_2	−	+	−	−	+	−
Nitrogen fixation	−	+	−	−	+	−
Nitrate reduction	−	−	+	−	−	+
Catalase	+	+	+	nd	+	+
Cytochrome oxidase	+	+	+	nd	+	+
Growth factors required:						
B_{12}				+		+
Biotin					+	
Thiamine					+	
Major quinone:						
MK-8					+	
Q-8	+	+	+	+	+	+
Habitats:						
Freshwater	+		+	+		+
Mud					+	
Soil		+			+	
Mol% G + C of DNA	66.2–66.3	69.1–71.1	68.1	67.8–71.1	70.0–72.5	69.9
Carbon source utilized:						
Acetate	d	−	+	−	+	+
Pyruvate	+	−	+	+	+	+
Lactate	+	+	+	−	+	+
Citrate	+	d	nd	−	+	+
Malate	+	+	nd	+	+	+
Succinate	+	+	+	+	+	+
D-Ribose	−	−	nd	+	nd	nd
D-Glucose	+	+	+	+	+	+
D-Fructose	+	+	+	−	+	+
D-Galactose	+	−	nd	nd	nd	+
Sucrose	−	+	nd	+	nd	+
Glycerol	−	+	nd	+	−	+
Mannitol	+	−	nd	nd	−	+
Sorbitol	−	−	nd	nd	−	+

[a]Symbols and abbreviations: +, 90% or more of strains positive; −, 90% or more of strains negative; d, 11–89% of strains positive; nd, not determined; PHB, poly-β-hydroxybutyrate.

by means of a single flagellum or several polar flagella. Motility is observed only in the early exponential phase of growth. Spores and sheaths are not formed. Cell suspensions and colonies are white to pink. Pigmentation varies depending upon growth media and culture age. Absorption maxima of living cells are at 482, 515, 550, 590, 800, and 870 nm. Bacteriochlorophyll *a* and carotenoids with spirilloxanthin as the major component are synthesized as photosynthetic pigments only under aerobic conditions in the dark.

Obligately aerobic chemoorganoheterotrophic bacteria producing photosynthetic pigments. Do not grow phototrophically under anoxic conditions in the light. Do not grow by anaerobic respiration with nitrate, dimethyl sulfoxide, or trimethylamine *N*-oxide as the terminal electron acceptors. Autotrophic growth with hydrogen is absent. Good carbon sources for growth are D-glucose, D-fructose,

D-galactose, mannitol, pyruvate, lactate, citrate, succinate, and L-malate. Good growth also occurs with Casamino acids, peptone, skim milk, and yeast extract. Most characteristic is the ability to co-metabolize and degrade aliphatic polycarbonates. Not utilized are D-ribose, sucrose, glycerol, and sorbitol. Hydrolytic activities against starch and gelatin are present. Acid production from glucose in Hugh–Leifson's OF medium is weak or negative. Oxidase and β-galactosidase (ONPG), but not catalase, are produced. Nitrate is not reduced to nitrite. Nitrogen fixation is negative. No growth factors are required.

Optimal growth occurs at 35°C (range: 5–43°C) and pH 6.5 (range: pH 5–8).

Habitat: wide variety of freshwater environments.

The mol% G + C of the DNA is: 66.2–66.3 (HPLC).

Type strain: Suyama 61A , DSM 11813.

GenBank accession number (16S rRNA): AB003623.

Genus Incertae Sedis XV. **Rubrivivax** Willems, Gillis and De Ley 1991b, 70[VP]

JOHANNES F. IMHOFF

Rub.ri.vi' vax. L. neut. adj. *rubrum* red; L. masc. adj. *vivax* long living; M.L. masc. n. *Rubrivivax* the red and long living (bacterium).

Cells are straight or curved rods, are motile by means of polar flagella, and multiply by binary fission. **Gram negative and belonging to the *Betaproteobacteria.*** Internal membrane systems are poorly developed or absent. **Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the spheroidene series. Ubiquinones and menaquinones with eight isoprene units (Q-8 and MK-8) are present. Straight-chain $C_{16:1}$ and $C_{16:0}$ are the major components of cellular fatty acids. $C_{10:0\ 3OH}$ is present.**

Photoautotrophic and photoheterotrophic growth may occur with hydrogen and a variety of carbon compounds as electron donors. **Chemotrophic growth is possible** by respiration under microoxic to oxic conditions in the dark or by fermentation. Cells grow well with simple organic compounds as electron donors and carbon sources, as well as in complex media containing peptone, yeast extract or Casamino acids. Growth factors are required. **Mesophilic and neutrophilic freshwater bacteria.** Habitat: freshwater ponds, sewage ditches, activated sludge.

The mol% G + C of the DNA is: 70.5–72.4 (Bd), 71.2–72.1 (HPLC).

Type species: **Rubrivivax gelatinosus** (Molisch 1907) Willems, Gillis and De Ley 1991b, 71 (*Rhodocyclus gelatinosus* (Molisch 1907) Imhoff, Trüper and Pfennig 1984, 341; *Rhodopseudomonas gelatinosa* (Molisch 1907) van Niel 1944, 98; *Rhodocystis gelatinosa* Molisch 1907, 22.)

FURTHER DESCRIPTIVE INFORMATION

Characteristic of *Rubrivivax gelatinosus* is the liquefaction of gelatin, which is catalyzed by an extracellular protease (Klemme and Pfleiderer, 1977). *R. gelatinosus* grows well with citrate as the carbon source and thereby excretes large amounts of acetate, which serves as the carbon source after citrate is exhausted (Schaab et al., 1972). Citrate lyase, the key enzyme for growth on citrate, has been characterized in this species (Giffhorn et al., 1972; Beuscher et al., 1974). *R. gelatinosus* can also be adapted to grow with CO as the sole energy and carbon source under anoxic conditions in the dark (Uffen, 1976). Under these conditions, the activities of ribulosebisphosphate carboxylase and enzymes of the serine pathway are enhanced (Uffen, 1983).

As with other phototrophic *Betaproteobacteria*, the major phospholipid components of *Rubrivivax gelatinosus* are cardiolipin, phosphatidylethanolamine, and phosphatidylglycerol (Imhoff and Bias-Imhoff, 1995). Straight-chain $C_{16:1}$ and $C_{16:0}$ acids are the main components of cellular fatty acids (see Table BXII.β.58 of the chapter on the genus *Rhodoferax*).

ENRICHMENT AND ISOLATION PROCEDURES

R. gelatinosus is widely distributed in freshwater habitats where purple nonsulfur *Bacteria* occur. Media for enrichment, isolation, and growth generally employed for freshwater purple nonsulfur *Bacteria* are also suitable for *Rubrivivax* species. Though citrate is not a commonly utilized substrate among purple nonsulfur

Bacteria, it can be used by several species. Nevertheless, it has been proven to be useful for the selective enrichment of *R. gelatinosus*. Standard techniques for the isolation of anaerobic bacteria in agar dilution series and on agar plates also can be applied for *Rubrivivax* (see Biebl and Pfennig, 1981; Trüper and Imhoff, 1992).

MAINTENANCE PROCEDURES

Rubrivivax species are readily maintained by standard procedures in liquid nitrogen. Preservation is also possible by lyophilization or storage at −80°C in a mechanical freezer.

DIFFERENTIATION OF THE GENUS *RUBRIVIVAX* FROM OTHER GENERA

Rubrivivax is clearly distinguished from other phototrophic purple bacteria by its separate phylogenetic line within the *Betaproteobacteria*. The high mol% G + C content of >70 is quite characteristic. Properties to differentiate *Rubrivivax* species from other phototrophic *Betaproteobacteria* are given in Tables 6 (p. 130) and 7 (p. 131) of the introductory chapter "Anoxygenic Phototrophic Purple Bacteria", Volume 2, Part A. The phylogenetic relationships of the phototrophic *Betaproteobacteria* are shown in Fig. 4 (p. 132) of that same chapter.

TAXONOMIC COMMENTS

Currently, only one species is known. *Rubrivivax gelatinosus* has been reported to occur in two distinct morphological forms (Biebl and Drews, 1969). Form I cells are clearly curved, 0.4–0.7 µm in diameter, and produce little slime during active growth. Form II cells are more or less straight rods, although they are sometimes also bent, and produce more slime during active growth, causing sedimentation of the cells in a gelatinous layer. Exemplified by two strains from each of the morphological groups, form I cells utilize a greater variety of carbon sources and have a much shorter doubling time than do form II cells (Weckesser et al., 1969). Another strain of this species, described by Klemme (1968), is intermediate in carbon substrate utilization with respect to the two groups established by Weckesser et al. (1969). Furthermore, the lipopolysaccharides of *R. gelatinosus* show two different serotypes, which do not cross-react with each other (Weckesser et al., 1975). It is not known whether correlations exist between serotypes, morphological types, and nutritional types. Detailed and careful studies are required to clarify the taxonomic status of the strains assigned to this species.

Further information is required on the specificity of gelatin liquefaction by *Rubrivivax gelatinosus*. Siefert et al. (1978) have identified about half of the strains with this property as belonging to *Rhodobacter capsulatus*, while other strains, with the morphology typical of *Rubrivivax gelatinosus*, do not liquefy gelatin. Weak hydrolytic activity of gelatin is also present in *Rhodoferax fermentans*.

List of species of the genus Rubrivivax

1. **Rubrivivax gelatinosus** (Molisch 1907) Willems, Gillis and De Ley 1991b, 71[VP] (*Rhodocyclus gelatinosus* (Molisch 1907) Imhoff, Trüper and Pfennig 1984, 341; *Rhodopseudomonas gelatinosa* (Molisch 1907) van Niel 1944, 98; *Rhodocystis gelatinosa* Molisch 1907, 22.)

ge.la.ti.no' sus. L. part. adj. *gelatus* frozen, stiffened; M.L. n.

gelatinum gelatin, that which stiffens; M.L. masc. adj. *gelatinosus* gelatinous.

Cells are rod shaped, straight, or slightly curved, 0.4–0.7 × 1–3 μm and in older cultures up to 15 μm long and irregularly curved. Most strains show abundant mucous production in all media, which causes the cells to clump together and appear immotile. In young cultures, cells are highly motile by means of polar flagella. Internal photosynthetic membranes appear as small fingerlike intrusions of the cytoplasmic membrane. Cultures grown anaerobically in the light are pale peach to dirty yellowish brown; aerobically grown cells appear colorless to light yellowish brown. Cells contain bacteriochlorophyll *a* (esterified with phytol) and carotenoids of the spheroidene series, with spheroidene, OH-spheroidene and spirilloxanthin as major components.

Preferably grow photoheterotrophically under anoxic conditions in the light, with a variety of organic compounds as electron and carbon sources. Photoautotrophic growth is also possible with hydrogen as electron source in the presence of growth factors. Chemotrophic growth is possible under microoxic to oxic conditions in the dark. Some strains can also adapt to grow anaerobically in the dark, with CO as sole carbon and energy sources. Pyruvate is fermented anaerobically in the dark. The carbon sources utilized are listed in Table BXII.β.59 of the chapter on the genus *Rhodoferax*. In addition, a variety of amino acids, yeast extract, and peptone are used. Most characteristic is the liquefaction of gelatin. Fatty acids are utilized only at low concentrations. Suitable nitrogen sources are ammonia, dinitrogen, and a number of amino acids; some strains may also utilize uracil, thymine, guanine, and uric acid both anaerobically in the light and aerobically in the dark, but xanthine and adenine only under oxic conditions. Sulfate can be used as the sole sulfur source. Biotin and thiamine are required as growth factors; some strains also require pantothenate.

Mesophilic freshwater bacterium, with optimal growth at 30°C and pH 6.0–8.5. Habitat: freshwater ponds, sewage ditches, and activated sludge. Major quinone components are Q-8 and MK-8.

The mol% G + C of the DNA is: 70.5–72.4 (Bd), 70.2–72.0 (T_m), 71.2–72.1 (HPLC); type strain, 71.9 (T_m).

Type strain: ATCC 17011, DSM 1709, LMG 4311.

GenBank accession number (16S rRNA): D16213, M60682.

Genus Incertae Sedis XVI. *Sphaerotilus* Kützing 1833, 386[AL]

PETER KÄMPFER AND STEFAN SPRING

Sphae.ro' ti.lus. Gr. n. *sphaera* a sphere; Gr. n. *tilus* anything shredded, floc, down; M.L. masc. n. *Sphaerotilus* spherical flock.

Straight rods, 1.2–2.5 × 2–10 μm, usually arranged in single chains within sheaths of uniform width, which may be attached by means of holdfasts to walls of containers, submerged plants, stones, and other surfaces. True, rather than false, branching of the filaments does not occur. **Single or paired cells released from the sheaths are motile by means of a bundle of subpolar flagella**, sometimes so intertwined as to give the appearance of a single large "unit flagellum". Sheaths usually thin without encrustation by ferric and manganic oxides. They cannot always be easily recognized when completely filled with cells, but if parts of the sheaths are vacated by the cells, recognition of the organism cannot be misinterpreted. Resting stages are not known. Gram negative. Has a propensity for storing **poly-β-hydroxybutyrate** in granules. **Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Can grow at very low concentrations of dissolved oxygen (below 0.1 mg/l). Temperature range: 10–40°C; optimum: between 20° and 30°C. pH range: 5.4–9; Optimal pH: between 6.5 and 7.5. **Chemoorganotrophic.** Alcohols, organic acids, and sugars are used as sources of carbon and energy. Ammonium salts and nitrates may serve as nitrogen source in the presence of vitamin B$_{12}$ or methionine. Peptone, Casamino acids, and mixtures of aspartic and glutamic acids, and vitamin B$_{12}$, or methionine give better results.

The mol% G + C of the DNA is: 70.

Type species: **Sphaerotilus natans** Kützing 1833, 386.

FURTHER DESCRIPTIVE INFORMATION

Pure cultures, upon prolonged incubation, may sometimes show large, circular bodies resembling protoplasts. Their formation is probably due to the production of enzymes involved in the decomposition of the cell walls during the death phase. Incorporation of 0.4 g glycine per liter of nutrient medium favors this phenomenon (Phaup, 1968).

Fig. BXII.β.55 illustrates partly filled and empty sheaths. The surface of the sheaths has a smooth structure, which is in contrast with the rough structure of the sheath surface of *Leptothrix* species (Fig. BXII.β.56). The sheaths of *S. natans* are covered with a cohering slime layer of variable thickness. For the composition of the slime, see Gaudy and Wolfe (1962). The composition of the sheath was investigated by Romano and Peloquin (1963), Petitprez et al. (1969), and Hoeniger et al. (1973). Takeda et al. (1998) found carbohydrate (54.1%), protein (12.2%), and lipid (1–3%) in the sheaths by colorimetric reactions and solvent extraction. Gas-liquid chromatography showed glucose and galactosamine to be present in the molar ratio of 1:4. The most abundant amino acids in the sheath protein were glycine (49.2 mol%) and cysteine (24.6 mol%). The sheaths were resistant to agents that reduce disulfide bonds (dithiothreitol and 2-mercaptoethanol) and to protease treatment. Sheaths could be degraded completely by hydrazine, and a heteropolysaccharide composed of glucose and galactosamine (1:4) was released. A specific enzyme produced by a *Paenibacillus* sp. acting on the polysaccharide moiety was described in detail by Takeda et al. (2000).

The size and shape of colonies depends largely on the ability of the respective strain to form sheaths. Typical colonies are rough (Fig. BXII.β.57). Often the colonies are characterized by a smooth central region and a "short-haired" periphery (Pellegrin et al., 1999). In broth cultures, the organisms often develop a pellicle on the broth surface composed of long, unbranched, sheathed filaments.

The normal habitat of *S. natans* is slowly running freshwater that is heavily contaminated with sewage or wastewater from pa-
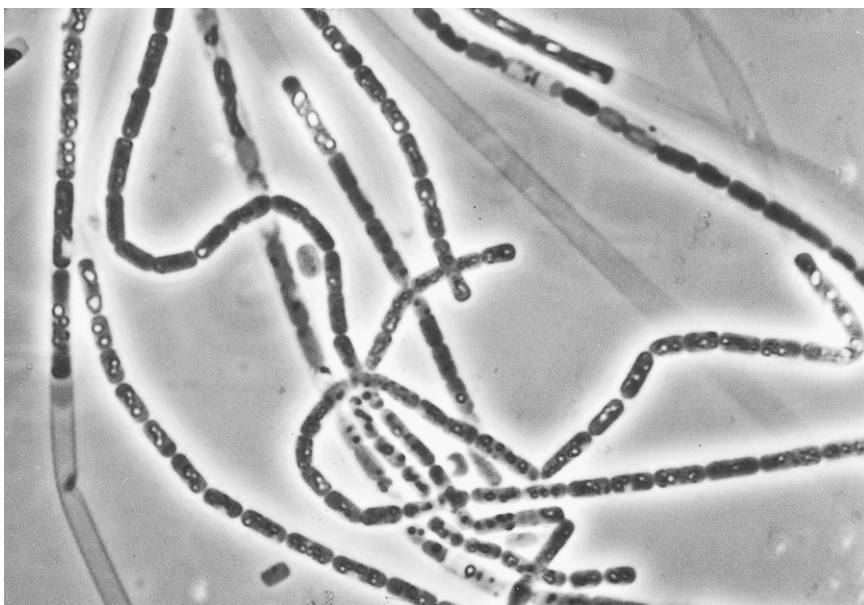
FIGURE BXII.β.55. *S. natans* filaments from a rough colony grown on agar medium containing glucose and peptone at 1 g/l each. Partly filled and empty sheaths can be seen. Many cells contain globules of poly-β-hydroxybutyrate. × 1006. (Reproduced with permission from E.G. Mulder and W.L. van Veen, Antonie van Leeuwenhoek Journal of Microbiology and Serology *29:* 121–153, 1963, ©Kluwer Academic Publishers, Dordrecht.)
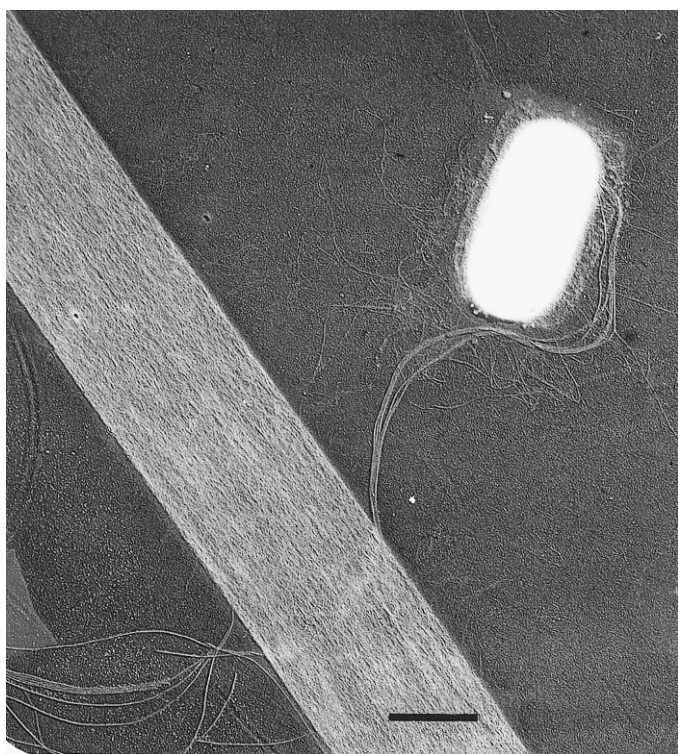
FIGURE BXII.β.56. Empty sheath of *S. natans* showing a smooth surface, and a single cell with a tuft of subpolar flagella. Electron micrograph. Bar = 1 μm. (Reproduced with permission from E.G. Mulder and M.H. Deinema. 1981. *In* Starr, Stolp, Trüper, Balows and Schlegel (Editors), The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bacteria. Springer-Verlag, Berlin, pp. 425–440.)
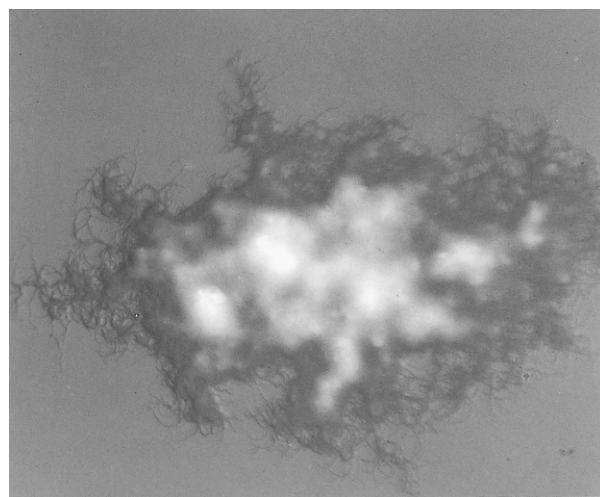
FIGURE BXII.β.57. Rough colony of *S. natans*. × 22. (Reproduced with permission from E.G. Mulder and W.L. van Veen, Antonie van Leeuwenhoek Journal of Microbiology and Serology. *29:* 121–153, 1963, ©Kluwer Academic Publishers. Dordrecht.)

per, potato, dairy, or other agricultural industries. The organism also occurs regularly in activated sludge, particularly when this material is settling poorly—so-called bulking. The relatively good growth under conditions of low oxygen concentrations and its capability to utilize a large number of organic carbon sources may be responsible for its relative dominance in biological deposits (Stokes, 1954; Dondero, 1975), including paper mill slimes (Väisänen et al., 1994; Pellegrin et al., 1999).

S. natans is one of several types of filamentous bacteria that may cause pipe clogging and bulking of activated sludge (Eikelboom, 1975). A ready settling of sludge flocs is one of the requisites for the successful operation of the activated-sludge process, which includes the aerobic biological purification of sewage and industrial wastewater. After absorption of the soluble wastes by the sludge organisms, the sludge flocs should readily settle, so that flocs and purified water can be separated. When the sludge flocs are densely populated by *S. natans* or some other filamentous organism, they are voluminous with many trichomes protruding into the surrounding water, thereby preventing a ready settling. This phenomenon is the cause of bulking sludge. Several different factors may cause the dominant growth of filamentous bacteria in activated sludges, among them: (a) low or high concentrations of available nutrients found in systems continuously fed with wastewater and (b) the low oxygen tensions that occur in such systems. With respect to the relatively high proportion of cell surface to cell contents of protruding filamentous bacteria compared with clumps of floc-forming bacteria, the former organisms occupy a more favorable position for nutrient uptake and growth. The ability of *S. natans* to thrive at very low pO_2 values is an additional factor favoring the competition with floc-forming bacteria. According to Wanner and Grau (1989), *Sphaerotilus* prefers environmental conditions with high sludge retention times and low concentrations of dissolved oxygen combined with high substrate concentrations. The organism does not often occur in anaerobic/aerobic selector plants. However, other factors may also lead to sludge bulking by other organisms. More details can be found in Eikelboom (1975), Jenkins et al. (1986), Wanner and Grau (1989), and Kämpfer (1997).

Although *S. natans* prefers a growth medium containing adequate amounts of easily assimilable organic nutrients, the organism is found sporadically in unpolluted water of brooklets, ditches, and ponds, where unknown compounds are the substrate. In the former habitat, the sheaths are thin and colorless; in the latter, particularly in the presence of soluble iron compounds, they may turn yellow-brown and sometimes become encrusted with ferric oxide. This characteristic can be clearly observed in a laboratory apparatus in which *S. natans* is grown in slowly running soil extract enriched with Fe (II). Under these conditions, the sheaths of *S. natans* resemble those of *Leptothrix ochracea*, and a number of authors (Pringsheim, 1949a, b; Stokes, 1954; and others) have assumed that both organisms are identical. However, 16S rRNA sequencing studies have shown the separate position of organisms belonging to both genera, although an unambiguous pattern of the respective branches of both genera is not possible on the basis of the available data set (Pellegrin et al., 1999), mainly because the 16S rRNA sequence of *L. ochracea*, which has not been cultivated in pure cultures until now, is not available.

Isoprenoid quinone Q-8 is the predominant quinone type for *S. natans* (Kämpfer et al., 1995, 1998). In a study of the fatty acid composition as determined by gas chromatographic analysis, the composition of 15 strains differed only slightly (Kämpfer, 1998). All strains contain the fatty acids $C_{12:0}$, $C_{16:0}$, $C_{16:1\ \omega7c}$, $C_{17:1}$, and $C_{18:1\ \omega7,\ 9c}$, and $C_{10:0\ 3OH}$; a fatty acid profile that is very similar to that of *Leptothrix* species and typical for members of the *Betaproteobacteria* (Table BXII.β.65).

ENRICHMENT AND ISOLATION PROCEDURES

Several isolation techniques for *Sphaerotilus* have been described. When slimy masses of the organism—attached to submerged surfaces in polluted, slowly running water—are available, direct isolation of *S. natans* may succeed. Pellegrin et al. (1999) collected slime samples from paper machines that were rinsed fivefold in sterile distilled water. The deposits were placed on the surface of FIL agar containing 0.005 g/l cycloheximide and incubated 72 h at 30°C. For details see Pellegrin et al. (1999). Rough colonies (Fig. BXII.β.57) were selected and further investigated. A similar procedure can be applied to activated sludge containing many filaments of the organism (i.e., bulking sludge). When the sheathed bacteria occur in low numbers in activated sludge or in nonpolluted water samples, the use of enrichment cultures may be desirable (Mulder and Deinema, 1981). Mulder (1989b) described a technique, based on extracted alfalfa straw (Stokes, 1954) or extracted pea straw (Mulder and van Veen, 1963, 1965), which can serve as the nutrient material. Most of the soluble organic matter should be removed to prevent the accumulation of undesirable organisms. This can be achieved by boiling and extracting the straw after it has been cut into pieces of about 2 cm. In the case of alfalfa straw, a 1% suspension is extracted three or four times by boiling with large amounts of tap water. The extracted straw medium is distributed in 50-ml quantities in 125-ml Erlenmeyer flasks, which are then inoculated with about 10 ml of water from various sources. The preparation of the pea straw medium differs slightly from the preceding technique. The straw is extracted for 10 h at 100°C with tap water that is renewed every hour. One or 2 g of extracted pea straw (dry weight) in 25 ml of tap water is autoclaved twice (15 min at 110°C) and used as an enrichment medium. After inoculation with small amounts of river or ditch water or activated sludge and subsequent incubation for about 1 week at 22–25°C, tufts of filaments of *S. natans* may be seen after microscopic observation.

Isolation may be achieved by streaking the enrichment cultures on previously dried agar plates containing low levels of nitrogen and carbon sources. Activated sludge containing many filaments of *S. natans* is streaked directly on such plates. Slimy masses of sheathed bacteria grown in slowly running, polluted water are washed several times with sterile water. Homogenization of the washed flocs by blending for a very short time may be advisable.

The use of a nutritionally poor agar medium limits the size

TABLE BXII.β.65. Main differential characteristics of the genera *Sphaerotilus* and *Leptothrix* [a]

Characteristic	*Sphaerotilus*	*Leptothrix*
Mn^{2+} oxidation[b]	−	+
F_2O_3 accumulation on the sheaths[b]	+	+
Reserve material: [c]		
Polysaccharide	+	−
Carbon sources used for growth (in GMBN base):[d]		
L-Alanine	+	−
L-Asparagine	+	−
L-Aspartate	+	−
Butyrate		+
D-Fructose	+	D
D-Glucose	+	D
D-Gluconate	+	−
L-Ornithine	+	−

[a]Symbols: see standard definitions.

[b]Data from Mulder (1989b).

[c]Data from Willems et al. (1991b).

[d]Data from Spring et al. (1996) and Kämpfer (1998). For additional results of carbon substrate utilization tests, see the references by these authors and also by Willems et al. (1991b).

of undesirable bacterial colonies, leaving large areas for the filamentous organisms. This medium has the following basal composition (per liter of glass-distilled water): KH_2PO_4, 27 mg; K_2HPO_4, 40 mg; $Na_2HPO_4 \cdot 2H_2O$, 40 mg; $CaCl_2$, 50 mg; $MgSO_4 \cdot 7H_2O$, 75 mg; $FeCl_3 \cdot 6H_2O$, 10 mg; $MnSO_4 \cdot H_2O$, 5 mg; $ZnSO_4 \cdot 7H_2O$, 0.1 mg; $CuSO_4 \cdot 5H_2O$, 0.1 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.05 mg; cyanocobalamin, 0.005 mg. This medium is enriched with peptone, 1 g/l; glucose, 1 g/l; and agar (Davis), 7.5 g/l. To inhibit the rapid spreading of contaminating bacteria, the excess surface moisture of the sterile agar plates should be evaporated by overnight storage of these plates at a temperature of 37–45°C. Upon inoculation and incubation of these plates at 20–25°C, colonies of *S. natans* may be seen and tentatively identified within a few days by their characteristically flat, dull, cotton-like appearance. The edges of the colonies are irregular, owing to curly filamentous growth extending in all directions (Fig. BXII.β.57). Confirmation of the identification may be achieved by microscopic observation (Fig. BXII.β.55).

Sphaerotilus may also be isolated by spread plate techniques with or without centrifugation or vortex treatment (Williams and Unz, 1985; Ziegler et al., 1990). Growth has been reported on I and SCY media (Eikelboom, 1975), SS media (Williams and Unz, 1985), R2A agar (Seviour et al., 1994), and GMBN-Agar (Kämpfer et al., 1995).

MAINTENANCE PROCEDURES

Stock cultures of *S. natans* on agar slants of the previously described media can be stored for about 3 months at 4°C. Addition of 2–3 ml of sterile tap water to the agar slants may prolong the viability for another 3 months. Preservation for longer periods is accomplished by common lyophilization techniques; however, it must be stressed that some *Sphaerotilus* strains do not survive lyophilization. Freezing in liquid nitrogen can be used for the long-term preservation of these strains.

DIFFERENTIATION OF THE GENUS *SPHAEROTILUS* FROM OTHER GENERA

Differential characteristics of the genera belonging to the *Rubrivivax gelatinosus–Leptothrix discophora* rRNA cluster of the *Betaproteobacteria* group are listed in Table BXII.β.66, and the main characteristics of the genera *Sphaerotilus* and *Leptothrix* are given in Tables BXII.β.65 and BXII.β.67. It can be seen that *Sphaerotilus* is closely related to *Leptothrix*. This applies to the motility of separate cells when released from the sheaths, to the formation of poly-β-hydroxybutyrate as reserve material, to the accumulation of ferric oxide on the sheaths, the quinone type and the fatty acid patterns, and to the mol% G + C of the DNA. However, several other properties are clearly different. They include morphological as well as physiological characteristics, such as size of cells; flagellation; structure of sheath surface; ability of the *Leptothrix* species to oxidize Mn^{2+} to Mn^{4+} (MnO_2), which is absent

in *Sphaerotilus*; and the pronounced response of *S. natans* to organic nutrients as contrasted with no or poor response of most *Leptothrix* species to added nutrients; the latter factor is especially of considerable ecological significance. *S. natans* thrives in water heavily contaminated with organic nutrients (wastewater); *Leptothrix* species are never found in such environments, except *L. cholodnii* and *L. lopholea*, which respond more clearly to added nutrients than do the other *Leptothrix* species. Pellegrin et al. (1999) found that *Sphaerotilus* isolates from paper-mill slimes were adapted to the specific environmental conditions, regarding temperature tolerance and utilization of cellulose and starch.

TAXONOMIC COMMENTS

The only known species of the genus is *S. natans*. The 16S rRNA gene sequences have been determined for several reference strains and isolates of *Sphaerotilus* (Corstjens and Muyzer, 1993; Siering and Ghiorse, 1996, 1997; Pellegrin et al., 1999) and are highly similar to each other (>99.5%). In phylogenetic trees, the highest similarities are found with *Leptothrix* species. Together with *Ideonella dechloratans*, *Rubrivivax gelatinosus*, *Alcaligenes latus*, *Roseateles depolymerans*, and *Aquabacterium* species (sequence similarities between 16S rRNA genes within this group are above 93%), they form a branch of bacteria that is phylogenetically distinct from the *Comamonadaceae* as defined by Willems et al. (1991b), and that is also referred to as the *Rubrivivax* subgroup of the *Betaproteobacteria* (Wen et al., 1999). A phylogenetic tree of members of the *Rubrivivax gelatinosus–Leptothrix discophora* rRNA cluster of the *Betaproteobacteria* is presented in Fig. BXII.β.58.

As with the genus *Leptothrix*, some characteristics that distinguish *Sphaerotilus natans* from *Leptothrix* species are not very stable and regularly lost upon cultivation. The ability to form sheaths has been lost by some strains available from public culture collections. In addition, it has been pointed out by Pellegrin et al. (1999) that based on comparison of 16S rDNA sequences, no unambiguous branching of *Sphaerotilus* and *Leptothrix* have been obtained with different treeing methods.

However, in view of the pronounced differences between *Sphaerotilus natans* and *Leptothrix* species, the genus allocation seems to be firm.

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List of species of the genus Sphaerotilus

1. **Sphaerotilus natans** Kützing 1833, 386[AL]

   na′ tans. L. part. adj. *natans* swimming.

   The cell morphology is as described for the genus. Loss of the sheath-forming capacity by mutation has been re-

ported (Stokes, 1954). Colonies of sheathless cells are smooth (they have lost their filamentous edges). Discontinuation of sheath formation may also be due to nongenetic factors, particularly nutritional conditions (Gaudy and

TABLE BXII.β.66. Characteristics of the genera belonging to the *Rubrivivax gelatinosus–Leptothrix discophora* rRNA cluster[a,b]

Characteristic	Rubrivivax	Ideonella	Roseateles	Leptothrix	Sphaerotilus	Pseudomonas saccharophila	Aquabacterium	Alcaligenes latus
Number of species	1	1	1	5[c]	1		3	
Flagellation	1, polar	2 or more, polar	2 or more, polar	1, polar	Tuft, subpolar	1, polar	1, polar	Peritrichous
Formation of sheaths	−	−	−	+	+	−	−	−
Photoautotrophic growth	+	−	−	−	−	−	−	−
Autotrophic growth with H_2	+	−	−			+	−	+
Oxidation of:								
Carbon monoxide	+							
Manganese (Mn^{2+})				+	−		−	
Accumulation of Fe_2O_3 on the sheaths				+	+			
Anaerobic growth with chlorate		+					−	
Nitrogen fixation	+		−			+	−	+
Gelatinase	+				Slow	+	−	+
Accumulation of:								
Poly-β-hydroxybutyrate			+	+	+	+	+	+
Polysaccharides				−	+		−	
Carotenoid pigments	+	−	+	−	−	−	−	−
Growth factors required:								
Biotin, thiamine	+	−	−	−	−			
Vitamin B_{12}	−	−	−	+[d]	+			
Carbon sources used for growth:								
Acetate	+	+	D	D	+	+	+	−
Pyruvate	+	+	+	D	+	+	D	−
Butyrate	D				+	+	+	+
Lacate	+	+	+	D	+	+	D	+
L-Malate	+		+	D	+	+	+	+
Succinate	+	+	+		+	+	+	+
Fumarate	+	+	D		+	+	+	+
Citrate	+		+		+	+	D	D
D-Ribose			−	D		+	−	−
Glucose	+		+	D	+	+[e]	−	+
Fructose			+	D			−	
D-Galactose			+	D	+	+	−	−
Sucrose			−	D	+	+	−	+
Glycerol	−		−	D	+	−	D	+
Mannitol	−		+	D	+	−	−	−
Sorbitol	−		−		+	−		−
Isolation source:								
Mud	+					+		
Sewage		+						
Soil								+
Water		+	+	+	+		+	
Mol% G + C content of DNA[f]	70.0–72.5	68.1	66.2–66.3	67.8–71.1	69.1	69.7	65–66	69.1–71.1

[a]Symbols: see standard definitions. Blank space, not determined or not applicable.

[b]Data adapted from Willems et al. (1991b), Malmqvist et al. (1994a), Spring et al. (1996), Kämpfer (1998), Kalmbach et al. (1999), and Suyama et al. (1999).

[c]Data for *Leptothrix lopholea* and *Leptothrix ochracea* are not available.

[d]Thiamine and biotin may be required by some strains (Mulder, 1989b).

[e]Growth may require mutation.

[f]Most G + C values were determined by use from thermal denaturation curves; the exception was the value for *Leptothrix cholodnii*, which was taken from Van Veen et al. (1978).

Wolfe, 1961; Mulder and van Veen, 1963). When the organism is grown on a basal medium with glucose and peptone at 1 g/l each, normal hairy colonies are formed (Fig. BXII.β.57), as contrasted with the smooth, almost circular colonies formed when 5 g of these nutrients are supplied. High concentrations of peptone are more effective in producing this effect than are sugars. Smooth colonies consist of sheathless cells that have larger dimensions than cells in sheaths. Transfer of the former cells to a poor medium restores sheath formation. Growth is supported on several media (see genus description).

False branching of the filaments occurs in every strain of *S. natans*, but it occurs in some strains more than others. Its occurrence depends on cultural conditions (relatively poor media) rather than on strain specificity (Pringsheim, 1949a).

Utilization of fructose, glucose, maltose, sucrose, lactate, pyruvate, and succinate as sole sources of carbon has been reported by Stokes (1954), Höhnl (1955), Mulder and van Veen (1963), Kämpfer (1998), and Pellegrin et al. (1999). Numerous other carbon sources can be utilized (for details see Kämpfer, 1998 and Pellegrin et al., 1999). Strains of *S.*

TABLE BXII.β.67. Other characteristics of the genera *Sphaerotilus* and *Leptothrix*[a]

Characteristic	Sphaerotilus	Leptothrix
Phylogenetic affiliation:		
Rubrivivax subgroup of the *Betaproteobacteria*	+	+
Mol% G + C of DNA[b,c]	70	68–71
Major quinone type	Q-8	Q-8
Major fatty acids:		
$C_{16:1}$	+	+
$C_{16:0}$	+	+
$C_{18:1\ \omega7,\ 9c}$	+	+
$C_{18:1}$	+	+
Hydroxyl fatty acid:		
$C_{10:0\ 3OH}$	+	+
Cell dimensions:[b]		
Width (μm)	1.2–2.5	0.6–1.5
Length (μm)	1–10	1.5–14
Reserve material: [b]		
Poly-β-hydroxybutyrate	+	+

[a]Symbols: see standard definitions.
[b]Data from Mulder (1989b).
[c]Data from Willems et al. (1991b).

natans differ widely in their capacity to utilize other carbon compounds. In contrast to most *Leptothrix* strains, *S. natans* utilizes relatively high concentrations of assimilable substrates, from which it synthesizes considerable amounts of cellular material.

Cells may contain large amounts of poly-β-hydroxybutyrate either as numerous small globules or as a few large globules. Polysaccharides may also accumulate. The synthesis of both reserve compounds is stimulated by a high carbon/nitrogen ratio in the medium (Mulder and van Veen, 1963) and by oxygen deficiency.

The major quinone is ubiquinone Q-8. Major fatty acids (>5%) are hexadecanoic acid, *cis*-9-hexadecenoic acid, and *cis*-9,11-octadecenoic acid. Minor fatty acids are 3-hydroxydecanoic acid, dodecanoic acid, octadecanoic acid, tetradecanoic acid, and *cis*-9,10-methyleneoctadecanoic acid.

The mol% G + C of the DNA is: 70 (T_m).
Type strain: ATCC 13338, DSM 6575.
GenBank accession number (16S rRNA): L33980.
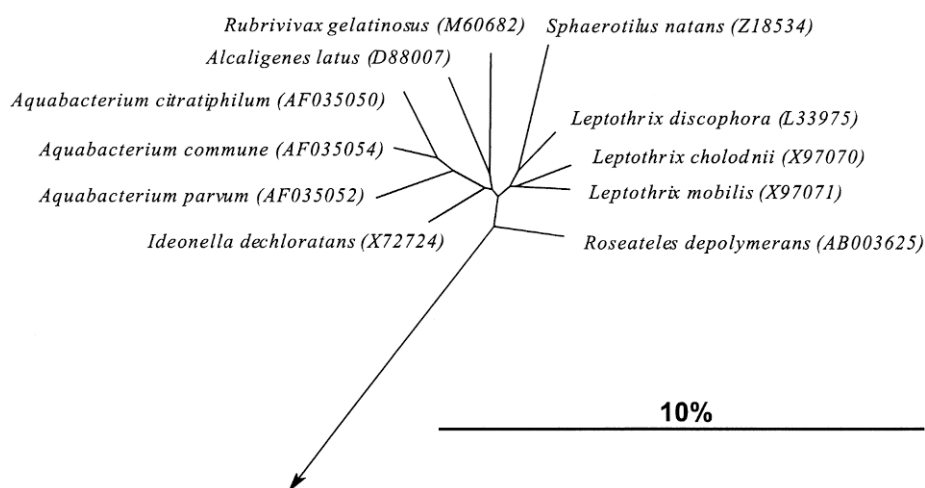
FIGURE BXII.β.58. Phylogenetic tree showing members of the *Rubrivivax gelatinosus–Leptothrix discophora* rRNA cluster of the *Betaproteobacteria*. This tree is based on a distance matrix analysis of almost complete sequences of 16S rRNA genes (at least 1300 nucleotides, GenBank accession numbers are given in parentheses). Phylogenetic distances were calculated as described by Felsenstein (1982). The sequence of *Escherichia coli* (L10328) was used as an outgroup (not shown). The scale bar represents 10% estimated sequence divergence.

Genus Incertae Sedis XVII. **Tepidimonas** *Moreira, Rainey, Nobre, da Silva and da Costa 2000, 741*[VP]

MILTON S. DA COSTA, M. FERNANDA NOBRE AND FRED A. RAINEY

Te.pi.di.mo' nas. L. adj. *tepidus* warm; Gr. n. *monas* unit; N.L. fem. n. *tepidimonas* warm monad.

Short rod-shaped cells 0.5–1.0 × 1.0–2.0 μm. Motile by means of one polar flagellum. Endospores are not formed. Cells stain Gram negative. Colonies are not pigmented. **Strictly aerobic.** Nitrate is not used as a terminal electron acceptor. Oxidase and catalase positive. **Optimal temperature for growth, between 50 and 55°C.** Optimal pH for growth, between 7.5 and 8.5. Fatty acids are of the straight-chain type. Major polar lipids are phosphatidylethanolamine and phosphatidylglycerol; ubiquinone 8 is the major respiratory quinone. **Chemolithoheterotrophic.** Reduced sulfur compounds are oxidized to sulfate in the presence of a metabolizable organic carbon source. Organic acids and amino acids are used as carbon and energy sources, but sugars, polysaccharides and polyols are not assimilated.

The mol% G + C of the DNA is: 69.7.
Type species: **Tepidimonas ignava** Moreira, Rainey, Nobre, da Silva and da Costa 2000, 741.

FURTHER DESCRIPTIVE INFORMATION

The optimal growth temperature of the type strain of *Tepidimonas ignava* is between 50 and 55°C; the organism does not

grow above 60°C or below 35°C. It is interesting to note that related strains ac-15 and DhA-73 (see Other Organisms, below) also have cardinal growth temperatures in this range and that the related environmental clone tmbr15-22 (see Other Organisms) was obtained from thermophilic aerobic treatment of synthetic wastewater. These results imply that the genus *Tepidimonas* contains several slightly thermophilic species with optimal growth rates in the neighborhood of 50°C.

The optimal pH for growth of *T. ignava* is between 7.5 and 8.5.

The polar lipid pattern, the respiratory quinones, and the fatty acids of this organism, are as expected for mesophilic or slightly thermophilic members of the *Betaproteobacteria*, although $C_{16:0}$, $C_{17:0}$, and $C_{18:0}$ are the predominant fatty acids.

Perhaps the most interesting characteristic of *T. ignava*—which is not restricted to this species—among the species of the *Betaproteobacteria* relates to its inability to assimilate any of the carbohydrates or polyols examined and to grow only on amino acids and organic acids. The inability to assimilate carbohydrates and polyols has also been reported for strain DhA-73, indicating that one or more steps of glycolysis are absent in both organisms. Strain DhA-73 is capable of degrading tricyclic diterpenes such as abietic acid, dehydroabietic acid, and palustric acid, found in pulp and paper mill effluent and derived from conifer resin (Yu and Mohn, 1999). The degradation of these acids was not examined in *T. ignava*. Strain ac-15 has never been characterized and comparisons between this organism and *T. ignava* cannot be made.[1]

Like several other members of the *Betaproteobacteria*, *T. ignava* oxidizes thiosulfate and tetrathionate to sulfate. However, this organism does not appear to be chemolithoautotrophic, since the oxidation of these reduced sulfur compounds occurs only in the presence of a metabolizable carbon source, indicating that it is chemolithoheterotrophic.

ENRICHMENT AND ISOLATION PROCEDURES

Only one strain of *T. ignava* was isolated from the hot spring at São Pedro do Sul in central Portugal, despite repeated attempts to isolate other strains of this organism. The isolation of this organism was achieved on a medium composed of one part of

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1. Recently a second species of the genus *Tepidimonas*, designated *Tepidimonas aquatica*, was described by Freitas et al. (2003). The new species has characteristics that are very similar to those of *T. ignava*. The major differences between the two species are in their fatty acid compositions. The 16S rRNA gene sequences of the two species have 97% similarity. This species and *T. ignava*, unlike strain DhA-73, do not degrade resinic acids.

Kligler's iron agar (Difco) and four parts of *Thermus* agar. Samples of water were filtered through cellulose nitrate membrane filters (pore diameter, 0.45 μm), placed on the surface of the Kligler's iron/*Thermus* medium, and incubated for several days at 50°C. However, the isolation of *T. ignava* appears to have been completely fortuitous, since it did not show any enhanced growth on this medium. The organism grows well on several low-nutrient media containing yeast extract and tryptone, namely *Thermus* medium (Williams and da Costa, 1992) and Degryse medium 162 (Degryse et al., 1978). The addition of thiosulfate (1.0–5.0 g/l) enhances growth of the organism.

MAINTENANCE PROCEDURES

Stock cultures of *T. ignava* remain viable for years at −80°C in *Thermus* medium containing 15% glycerol. Cultures have also been maintained lyophilized for several years without the loss of viability.

DIFFERENTIATION OF THE GENUS *TEPIDIMONAS* FROM OTHER GENERA

The bacteria of the genus *Tepidimonas* can be easily distinguished from all members of the family *Comamonadaceae* because of their high growth temperature range. Other characteristics, namely the polar lipids and the fatty acids, are similar.

TAXONOMIC COMMENTS

Phylogenetic analysis of the 16S rRNA gene sequence (Fig. BXII.β.59) shows that *Tepidimonas ignava* clusters within the class *Betaproteobacteria*, but represents a distinct lineage along with other undescribed strains, namely strain ac-15, isolated some years ago from a hot spring microbial mat at Yellowstone National Park, USA (EMBL accession number U46749; Nold et al., 1996), and strain DhA-73 (EMBL accession number AF125877; Yu and Mohn, 1999), isolated from a bioreactor treating bleached kraft mill effluent. An environmental 16S rRNA gene clone, designated tmbr15-22 (EMBL accession number AF309815) also belongs to this lineage. *Tepidimonas ignava* shares 96.2% 16S rRNA gene sequence similarity with strain ac-15, 97.5% sequence similarity with strain DhA-73, and 97.4% sequence similarity with the environmental clone tmbr15-22. These results indicate that the undescribed organisms (strains ac-15 and strain DhA-73) and the organism detected as the environmental clone tmbr15-22 represent novel species within the genus *Tepidimonas*. Moreover, this group shares a low 16S rRNA gene sequence similarity (<94.0%) with any previously described species within the *Betaproteobacteria*, and can be considered to represent a distinct lineage of, at least, genus status within the family *Comamonadaceae*.

List of species of the genus Tepidimonas

1. **Tepidimonas ignava** Moreira, Rainey, Nobre, da Silva and da Costa 2000, 741[VP]

ig.na′va L. adj. *ignavus* lazy, pertaining to the organism's trait of not using sugars for growth.

The characteristics are as described for the genus, with the following additional information. Colonies on Degryse 162 medium are not pigmented and are 1–2 mm in diameter after 60 h of incubation. Growth occurs above 35°C and below 65°C. Growth does not occur at pH 6.0 or pH 10.0. The major fatty acid is $C_{16:0}$; unsaturated fatty acids are also present in large amounts. Ubiquinone 8 is the major respiratory quinone. Yeast extract, or growth factors,

are required for growth. Nitrate is not reduced to nitrite. Thiosulfate is oxidized to sulfate and serves as an energy source coupled to the assimilation of organic substrates. Chemolithoheterotrophic growth occurs on reduced sulfur compounds. Xylan, starch, casein, elastin, and fibrin are not degraded. Several amino acids and organic acids are utilized for growth, but hexoses, disaccharides, pentoses, and polyols are not used.

Isolated from the hot spring at São Pedro do Sul in central Portugal.

The mol% G + C of the DNA is: 69.7 (HPLC).
Type strain: SPS-1037, DSM 12034.
GenBank accession number (16S rRNA): AF177943.

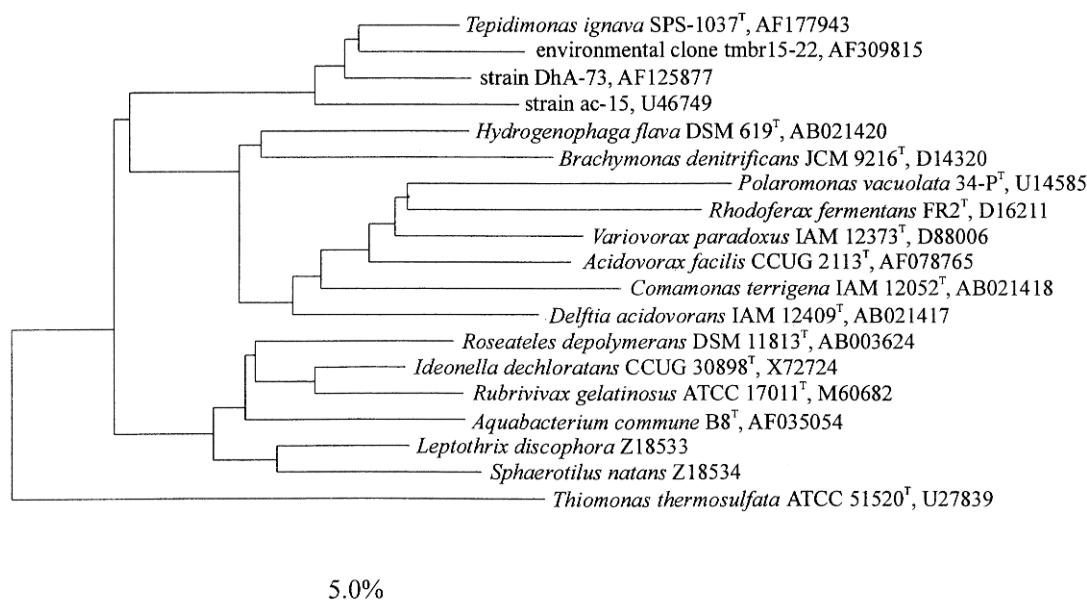**FIGURE BXII.β.59.** Phylogenetic dendrogram based on 16S rRNA gene sequence comparisons indicating the position of the genus *Tepidimonas* within the radiation of genera considered to represent the family *Comamonadaceae*. The scale bar represents 5 inferred nucleotide substitutions per 100 nucleotides.

Other Organisms

Strain ac-15 (EMBL accession number U46749) was isolated from a hot spring microbial mat at Yellowstone National Park, USA, by Nold et al. (1996). *Tepidimonas ignava* shares 96.2% 16S rRNA gene sequence similarity with strain ac-15.

Strain DhA-73 (EMBL accession number AF125877) was isolated from a bioreactor treating bleached kraft mill effluent by Yu and Mohn (1999). *Tepidimonas ignava* shares 97.5% sequence similarity with strain DhA-73.

The environmental 16S rRNA gene clone tmbr15-22 (EMBL accession number AF309815) was obtained from the thermophilic aerobic treatment of synthetic wastewater. *Tepidimonas ignava* shares 97.4% sequence similarity with strain tmbr15-22.-

Genus Incertae Sedis XVIII. **Thiomonas** Moreira and Amils 1997, 527[VP]

DONOVAN P. KELLY AND ANN P. WOOD

Thi.o.mo' nas. Gr. n. *thios* sulfur; Gr. fem. n. *monas* a unit, monad; M.L. fem. n. *Thiomonas* sulfur monad.

The phenotypic description is that of the general traits for group II of *Thiobacillus* (Kelly and Harrison, 1989; Moreira and Amils, 1997). Gram-negative, nonsporeforming, short rods that are about 0.3–0.5 × 1–3 μm. Cells motile by means of single polar flagella. Obligate aerobes. Optimal temperature 30–36°C for mesophilic species and 50°C for moderately thermophilic species. Optimal pH between 3 and 6. Facultative chemolithoautotrophs; optimal growth occurs in mixotrophic media supplemented with reduced sulfur compounds and organic supplements (yeast extract, peptone, some sugars, and amino acids). Chemoorganotrophic growth on yeast extract, Casamino acids, peptone, and meat extract. Chemolithoautotrophic on thiosulfate, tetrathionate, S^0, and H_2S. Do not oxidize ferrous iron. Sensitive to ampicillin. Contain ubiquinone Q-8. Members of the *Betaproteobacteria*.

The mol% G + C of the DNA is: 61–69.

Type species: **Thiomonas intermedia** (London 1963) Moreira and Amils 1997, 527 (*Thiobacillus intermedius* London 1963, 335.)

FURTHER DESCRIPTIVE INFORMATION

This genus was created by Moreira and Amils (1997), who determined complete sequences of the 5S and 16S rRNA genes for *Thiobacillus cuprinus* and compared them with homologous sequences from *Thiobacillus intermedius*, *Thiobacillus perometabolis*, and *Thiobacillus thermosulfatus*. These four species were found to form a phylogenetic cluster within the *Betaproteobacteria*, but to be only remotely related to the type species of *Thiobacillus*, *Thiobacillus thioparus*. The properties of the species of *Thiomonas* are summarized in Table BXII.β.68. All the species are moderately acidophilic and facultatively chemolithotrophic, with *Thiomonas cuprina* also able to grow autotrophically on pyrite (Huber and Stetter, 1990; Moreira and Amils, 1997). The properties, taxonomy, and differentiation of the genera of sulfur-oxidizing, che-

TABLE BXII.β.68. Basic characteristics of species of the genus *Thiomonas*

Characteristic	*T. intermedia*	*T. cuprina*	*T. delicata*	*T. perometabolis*	*T. thermosulfata*
Mol% G + C	65–67	67–68	66–67	65–66	61
Cell size (μm)	0.6–0.8 × 1.0–1.4	0.3–0.5 × 1.0–4.0	0.4–0.6 × 0.7–1.6	0.4–0.5 × 1.1–1.7	0.9 × 1.3–2.3
Motility	+	+	−	+	+
Carboxysomes	+	nd	nd	−	nd
Facultatively chemolithoautotrophic[a]	+	+	+	+	+
Growth on complex media	+	+	+	+	+
Optimal pH	5.5–6.0	3.0–4.0	5.5–6.0	5.5–6.0	5.2–5.6
pH limits	5.0–7.5	2.0–6.5	5.0–7.0	5.0–7.0	4.3–7.8
Optimal temperature, °C	30–35	30–36	30–35	35–37	50–53
Nitrate reduction to N_2	−	−	−[b]	−	−
Growth on:					
Sulfur	+	+	+	+	+
Thiosulfate	+	−	+	+	+
Tetrathionate	+	−	+	+	+
Trithionate	nd	−	nd	nd	nd
Metal sulfide ores[c]	−	+	nd	−	nd
Thiocyanate	−	−	−	−	−

[a]Optimal growth is mixotrophic in media with reduced sulfur compounds plus yeast extract, peptone, some sugars and amino acids.

[b]Nitrate reduced to nitrite.

[c]Chalcopyrite, arsenopyrite, galena, sphalerite.

molithoautotrophic, Gram-negative, rod-shaped bacteria are summarized in Tables BXII.β.70 and BXII.β.71 and Fig. BXII.β.60 in the chapter on the genus *Thiobacillus*. *T. cuprina* (DSM 5495) contains a circular chromosome (3.8 Mb) and appears to contain a linear 50 Mb megaplasmid that is inducible during chemolithotrophic growth (Marín et al., 1995; Amils et al., 1998).

List of species of the genus Thiomonas

1. **Thiomonas intermedia** (London 1963) Moreira and Amils 1997, 527[VP] (*Thiobacillus intermedius* London 1963, 335.)
   in.ter.me′ di.a. L. prep. *inter* between, among; L. adj. *media* middle; M.L. adj. *intermedia* in between, intermediate.

   Thin, short rods, 0.6–0.8 × 1.0–1.4 μm. Motile by means of a polar flagellum. On thiosulfate agar, small colonies (up to 1 mm) with raised centers develop that are yellowish and opaque with precipitated sulfur and surrounded by veil-like fringes. This organism is facultatively mixotrophic. Capable of chemolithotrophic, autotrophic growth on sulfur, thiosulfate, or tetrathionate, but not on thiocyanate. Also oxidizes sulfide. Aerobic; unable to denitrify. Unable to grow in heterotrophic media such as nutrient broth or with single organic substrates in the absence of thiosulfate. Grows very poorly on yeast extract alone but produces substantial growth (after lags of 1–10 d) on yeast extract supplemented with glucose, fructose, sucrose, maltose, aspartate, or glutamate. It is possible that a reduced sulfur compound stimulates growth under these conditions. Best growth is with mixotrophic media containing thiosulfate and yeast extract, alanine, malate, succinate, citrate, 2-oxoglutarate, serine, lactate, or the supplements listed for heterotrophic growth. This organism can use ammonium salts, nitrate, urea, glutamate, or aspartate as nitrogen sources. Optimal temperature: 30–35°C; growth range: 15–37°C. Optimal pH: 5.5–6.0; growth range pH: 5.0–7.5, although for mixotrophic media, the pH may be lowered to about 2.8. Isolated from freshwater mud; presumably widely distributed. DNA hybridization with *Thiomonas perometabolis* has been reported as 31–35 (Katayama-Fujimura et al., 1983) and 56–78 (Harrison, 1983).

   The mol% G + C of the DNA is: 65–67 (T_m).
   Type strain: ATCC 15466.

2. **Thiomonas cuprina** Moreira and Amils 1997, 527[VP]
   cu.pri′ na. L. adj. *cuprina* copper, describing its ability to extract copper ions from ores.

   Cells are Gram-negative rods, about 1–4 × 0.3–0.5 μm. Each is motile by one polar flagellum. Colonies on agar plates (yeast extract medium) have a brownish color. Optimal temperature 30–36°C, no growth at 15 or 50°C. Optimal pH 3–4, no growth at pH 1 or 7.5. Facultative chemolithotroph, aerobic. Chemoorganotrophic growth on yeast extract, Casamino acids, peptone, meat extract; some strains grow on pyruvate. Chemolithoautotrophic growth on chalcopyrite, sphalerite, arsenopyrite, galena, S^0, and H_2S, forming sulfuric acid. No oxidation of ferrous iron. Sensitive to ampicillin; *meso*-diaminopimelic acid present. Contain ubiquinone Q-8 but no rusticyanin. Lives in continental solfataric fields and mines. Insignificant DNA hybridization with *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Thiobacillus thioparus*, *Thiobacillus neapolitanus*, *Thiobacillus prosperus*.

   The mol% G + C of the DNA is: is 66–69 (T_m, HPLC).
   Type strain: DSM 5495.
   GenBank accession number (16S rRNA): U67162.

3. **Thiomonas perometabolis** (London and Rittenberg 1967) Moreira and Amils 1997, 527[VP] (*Thiobacillus perometabolis* London and Rittenberg 1967, 218.)
   pe.ro.me.ta′ bo.lis. Gr. adj. *peros* maimed, crippled; Gr. v. *metabole* alter, change; M.L. part. adj. *perometabolis* with a maimed metabolism.

   Thin, short rods with rounded ends, 0.4–0.5 × 1.1–1.7 μm. Motile by means of a polar flagellum. Colonies grown on yeast extract–thiosulfate agar (1–3 mm after 1 week) are circular, entire, convex, smooth, creamy white,

and opaque; the center of old colonies becomes pink-orange. Colonies grown on thiosulfate agar (0.5 mm after 10 d) are circular, entire, convex, smooth, creamy white, and opaque, developing a brown center with age. The original isolate of *T. perometabolis* did not exhibit chemolithotrophically autotrophic or heterotrophic growth on single-carbon substrates (London and Rittenberg, 1967), and its "maimed metabolism" was described as "obligately mixotrophic" (Vishniac, 1974). Further study led to an emended description (Katayama-Fujimura and Kuraishi, 1983; Katayama-Fujimura et al., 1984a) on which this entry is based. Facultative chemolithoautotroph. Chemolithotrophic autotrophic growth occurs on thiosulfate, tetrathionate, or sulfur, but not on thiocyanate. Little or no tetrathionate or trithionate accumulates during growth on thiosulfate. This organism exhibits diauxic growth on a mixture of thiosulfate and glutamate, with preferential use of the thiosulfate. Grows slowly after a lag time of about 2 weeks in heterotrophic media containing one of the following: alanine, glutamate, aspartate, malate, citrate, or succinate. Lags are shortened by the presence of thiosulfate. Best growth occurs in mixotrophic media with thiosulfate and organic supplements, such as yeast extract, casein hydrolysate, 2-oxoglutarate, some sugars, and some amino acids. Probably requires a reduced inorganic sulfur compound during heterotrophic growth. Obligate aerobe. Ammonium salts, nitrate, and urea are used as nitrogen sources, and glutamate and aspartate are used as both carbon and nitrogen sources. Optimal temperature: 35–37°C; growth range: 15–42°C. Optimal pH: 5.5–6.0; growth range pH: 5.0–7.0, although for mixotrophic media, the pH is lowered to about 2.8. Isolated

from soil. Distribution unknown. DNA hybridization with *T. intermedia* has been reported as both 31–35% (Katayama-Fujimura et al., 1983) and 56–78% (Harrison, 1983).

The mol% G + C of the DNA is: 65–66 (T_m).
Type strain: ATCC 23370.

4. **Thiomonas thermosulfata** (Shooner, Bousquet and Tyagi 1996) Moreira and Amils 1997, 527[VP] (*Thiobacillus thermosulfatus* Shooner, Bousquet and Tyagi 1996, 414.)
ther.mo.sul.fa' ta. Gr. n. *thermus* heat; L. n. *sulfatus* sulfur; L. adj. *thermosulfata* organism that produces sulfate and grows at high temperatures.

Cells are Gram-negative rods, 0.9 × 1.3–2.3 µm, motile by means of single polar flagella. Typically, cells contain 2–3 polyphosphate inclusions, and polyhedral bodies. Colonies on thiosulfate agar are small (<1 mm), and round and either are translucent or have sulfur deposits in the center. Strictly aerobic; grows chemolithoautotrophically on thiosulfate, tetrathionate, and sulfur, and chemoorganotrophically on yeast extract, succinate, and glutamate. Tetrathionate, trithionate, and sulfur are produced during growth on thiosulfate. Autotrophic growth on thiosulfate occurs between pH 4.3–7.8 (optimal pH 5.2–5.6) and 34–65°C (optimal 50–52.5°C). pH should be decreased to 2.5 on thiosulfate and sulfur media. Does not grow on carbohydrates, pyruvate, acetate, or formate; does not denitrify. Cells adhere to S^0 by means of a glycocalyx. Contains ubiquinone Q-8.

The mol% G + C of the DNA is: 61 (UV ratios).
Type strain: ATCC 51520.
GenBank accession number (16S rRNA): U27839.

Other Organisms

1. *Thiomonas delicata comb. nov.* (*Thiobacillus delicatus* Katayama-Fujimura, Kawashima, Tsuzaki and Kuraishi 1984a, 142.)
del.i.cat' a. L. adj. *delicata* delicate.

Rods, usually single, rarely in pairs, 0.4–0.6 × 0.7–1.6 µm. Nonmotile. Colonies grown on yeast extract–thiosulfate agar (1 mm in diameter) are smooth and circular and change from transparent to whitish-yellow with sulfur. Facultative chemolithotroph and mixotroph. Grows autotrophically with sulfur, thiosulfate, or tetrathionate, but not with thiocyanate; accumulates tetrathionate and trithionate transiently during growth on thiosulfate. Incapable of heterotrophic growth on single carbon compounds. Grows mixotrophically in thiosulfate media supplemented with tricarboxylic acid cycle intermediates or amino acids. Optimum growth requires both organic substances and thiosulfate or sulfur. Facultative anaerobe; reduces nitrate and produces nitrite in mixotrophic and autotrophic media with

thiosulfate or tetrathionate. Ammonium salts, nitrate, urea, glutamate, or aspartate can be used as nitrogen sources. Optimal temperature: 30–35°C; growth range: 15–42°C (no growth at 10°C or 45°C). Optimal pH: 5.5–6.0; growth range: pH 5.0–7.0. Isolated from mine water. Distribution unknown.

The mol% G + C of the DNA is: 66–67 (T_m, chemical analysis).
Deposited strain: THI 091, IAM 12624.
Additional Remarks: This species cannot yet be firmly assigned to the genus *Thiomonas*, but its G + C content, mixotrophy, and physiological similarities to *T. perometabolis* indicate that it should be reassigned to *Thiomonas* (Y. Katayama, personal communication). Determination of its 16S rRNA sequence is essential to confirm its phylogenetic relationships (Kelly and Harrison, 1989; Moreira and Amils, 1997).

Genus Incertae Sedis XIX. **Xylophilus** *Willems, Gillis, Kersters, Van Den Broecke and De Ley 1987, 428[VP]*

ANNE WILLEMS AND MONIQUE GILLIS

Xy.lo' phi.lus. Gr. n. *xylon* wood; Gr. n. *philos* friend; M.L. masc. *Xylophilus* friend of wood.

Straight to slightly curved rods, 0.4–0.8 × 0.6–3.3 µm, occurring singly, in pairs or short chains. **Long filamentous cells may occur in older cultures** (length may be 30 µm or more). **Gram negative.**

Motile by a single polar flagellum. Aerobic, having a strictly respiratory type of metabolism with oxygen as the only terminal electron acceptor. *In vitro* **growth is generally very slow** and rather

poor, even at the optimal growth temperature of 24°C. **Oxidase negative**, catalase positive. **Chemoorganotrophic** with oxidative carbohydrate metabolism. *Xylophilus* causes bacterial necrosis and canker of grapevine and can be isolated from infected plant material.

The mol% G + C of the DNA is: 68–69.

Type species: **Xylophilus ampelinus** (Panagopoulos 1969) Willems, Gillis, Kersters, Van Den Broecke and De Ley 1987, 428 (*Xanthomonas ampelina* Panagopoulos 1969, 75.)

FURTHER DESCRIPTIVE INFORMATION

As the genus contains only one species, all of the characteristics provided below describe the species *Xylophilus ampelinus*.

Colonial characteristics On nutrient agar, colonies are circular, semitranslucent, slightly raised, glistening, and pale yellow with entire margins; colony diameters are 0.2–0.3 mm after 6 d and 0.6–0.8 mm after 15 d. Better growth is obtained on GYCA medium[1] and best growth occurs on YGC medium[2]. On the latter medium colonies are yellow and a brown diffusible pigment is produced. *Xylophilus* strains may produce two stable colony types with one type (t1) consisting of relatively large yellow colonies (diameter 0.8–2.0 mm after 15 d on GYCA) and the other (t2) consisting of smaller, paler and more slowly growing colonies (diameter 0.4–1.0 mm after 15 d). Both types were highly similar microscopically and when analyzed by whole-cell protein gel electrophoresis and by DNA–DNA hybridization (Willems et al., 1987).

Pigments The yellow pigments produced by *Xylophilus* strains are different from xanthomonadins, the yellow water-soluble brominated aryl-polyene pigments produced by another plant-pathogenic genus, *Xanthomonas*. *Xylophilus* pigments appear sensitive to potassium isobutoxide but are generally very hard to purify and therefore little is known about them (Starr et al., 1977).

Growth conditions Minimum and maximum growth temperature are 6°C and 30°C, respectively (Panagopoulos, 1969). Optimal growth is obtained at 24°C. In a medium consisting only of a salt solution, ammonium chloride, and glucose or galactose, addition of 0.1% glutamic acid is required for growth (Panagopoulos, 1969).

Nutrition and metabolism *Xylophilus* strains have a strictly aerobic chemoorganotrophic metabolism. They use only a limited number of carbohydrates, organic acids, and amino acids for growth. In a study on the use of 60 substrates by nine French *Xylophilus* isolates, growth was observed on only D-glucose, D-galactose, L-glutamic acid, Na-succinate, Na-fumarate, K,Na-tartrate, Na-L-malate, Na₃-citrate, and Ca-gluconate (Van den Mooter and Swings, 1990). In general, *Xylophilus* strains show little variation from one another: strains from different geographic origin were shown to be highly similar by means of whole-cell protein gel electrophoresis, comparison of 106 enzymatic features and DNA–DNA hybridization (Willems et al., 1987). However, certain physiological characteristics do show considerable variation: the use of glucose and tartaric acid for growth and tyrosinase activity may vary between different populations (Ridé, 1996). Metabolic fingerprints using Biolog® GN plates

were produced for a limited number of strains, but the recommended sucrose peptone agar for preparation of cell cultures was replaced by nutrient agar. Under these conditions the strains oxidized only acetic acid, propionic acid, L-aspartic acid, L-glutamic acid, and L-pyroglutamic acid (Serfontein et al., 1997).

Biosynthesis of aromatic amino acids A study of the regulation mechanisms involved in the biosynthesis of aromatic amino acids has shown that 3-deoxy-D-arabinoheptulonate-7-phosphate synthetase is inhibited by tryptophan, chorismate, prephenate, phenylalanine, and tyrosine. Co^{2+} is needed for maximum activity. Prephenate-dehydrogenase is NAD^+-specific and is not inhibited by tyrosine (Whitaker et al., 1981; Byng et al., 1983).

Chemotaxonomic characteristics *X. ampelinus* contains putrescine as the main polyamine and smaller amounts of 2-hydroxyputrescine, spermidine, and spermine (Auling et al., 1991).

Phage susceptibility Various bacteriophages have been isolated from soil or water near infected vines and are differentiated by the morphology of their plaques on exponentially growing plates of *Xylophilus* strains. All strains of *Xylophilus* are sensitive to phage P *X.a.*15, which produces plaques that keep expanding over several days with the formation of a halo. Other phages are specific to certain groups of strains (Ridé, 1996).

Antibiotic sensitivity Nine *Xylophilus* strains tested were susceptible to 16 of 20 antibiotics tested (see Table BXII.β.69) and all nine strains were resistant to methicillin. Mixed results were obtained for ampicillin, colistin sulfate, and polymyxin B (Van den Mooter and Swings, 1990).

Pathogenicity *Xylophilus* strains are plant pathogens. They are responsible for bacterial necrosis and canker of grapevine (*Vitis vinifera*) in the Mediterranean region and South Africa (Panagopoulos, 1969; Erasmus et al., 1974). Although first described in France in 1895 as "Maladie d' Oléron" (Ravaz, 1895), the causal agent of bacterial necrosis of grapevine was not isolated until 1969 (Panagopoulos, 1969). The disease becomes apparent in early spring, when buds on affected shoots fail to open. Longitudinal cracks and cankers appear as they develop from hyperplasiae in the cambial tissue. Underlying vascular tissue shows a brown discoloration and will eventually die. Other parts of the plant that may be infected include petioles, flower stalks, and fruit stalks, resulting in death of leaves, flowers, or fruits. Leaves infected through hydathodes or stomata will show reddish-brown lesions. Roots can also be affected, resulting in retarded growth of shoots. Severity of symptoms may vary considerably for different varieties of grapevine (Panagopoulos, 1969; Grasso et al., 1979; Ridé, 1984, 1996; López et al., 1987). From being a rather rare disease at the beginning of the 20th century, it has now become more significant in the Mediterranean area through a combination of factors such as favorable environmental conditions, pruning procedures, and the increased mechanization of various viticultural practices (Ridé, 1996).

An *in vitro* test to assess cultivar susceptibility has been described by Peros et al. (1995). It involves inoculation of 2-month-old plantlets (having 8–12 internodes) by decapitation with scissors dipped in a bacterial suspension. After several weeks of incubation, the number of internodes with symptoms is recorded as an estimate for the progression of infection.

ENRICHMENT AND ISOLATION PROCEDURES

Isolation from affected vines is possible year round. The extremely slow and poor growth of *Xylophilus* strains often com-

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1. GYCA medium (g/l distilled water): yeast extract, 5.0; glucose, 10.0; CaCO₃, 30.0; and agar, 20.0.

2. YGC medium (g/l distilled water): yeast extract, 10.0; galactose, 20.0; CaCO₃, 20.0; and agar, 20.0.

TABLE BXII.β.69. Characteristics of *Xylophilus ampelinus*[a,b]

Characteristic[c]	Reaction
Hydrolysis of Tween 80	+
Hydrolysis of starch, tributyrin	d
Polygalacturonase, tyrosinase	d
Urease, catalase	+
Alkaline reaction in litmus milk (18 days)	+
Acetoin	d
Growth at 28°C	+
Growth at pH 6.5	d
Tolerance to 1% and 0.5% NaCl	d
Tolerance to 0.01% actidione on GYCA medium	+
Tolerance to 2,3,5-triphenyltetrazolium chloride (TTC) at concentrations of 0.005%, 0.01%, and 0.02%	d
Production of H$_2$S from thiosulfate	+
Production of H$_2$S from L-cysteine, peptone	d
Acid production from L-arabinose	+
Acid production from D-galactose, glycerol, maltose	d
Growth on L-arabinose, acetate (0.2%), L-glutamic acid, sodium L-malate, trisodium citrate, potassium sodium tartrate	+
Growth on D-glucose, D-galactose, sodium succinate, sodium fumarate, calcium gluconate, calcium lactate	d
Growth on 0.5% yeast extract	+
Growth on a medium containing NH$_4$Cl, D-glucose, mineral salts, and vitamins	+
L-Glutamate (0.1%) required for growth	+
Stimulatory effect on growth in basal medium by:	
L-Asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, L-serine, L-tryptophan	+
L-Alanine, L-arginine, glycine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-threonine, L-tyrosine, L-lysine, L-valine, and L-ornithine	d
Glycine and L-lysine as sole nitrogen sources	d
L-Glutamic acid as sole nitrogen and carbon source	+
L-Glutamine as sole source of nitrogen and carbon	d
Hydrolysis of the following substrates in API enzymatic tests:[d]	
2-Naphthyl-butyrate, 2-naphthyl-caprylate, 2-naphthyl-valerate, 2-naphthyl-caproate, 2-naphthyl-phosphate (pH 5.4), L-leucine-naphthylamide, L-tyrosine-naphthylamide, L-serine-naphthylamide, L-lysine-naphthylamide, glycine-naphthylamide, L-phenylalanine-naphthylamide, L-aspartic acid-naphthylamide, L-arginine-naphthylamide, L-alanine-naphthylamide, DL-methionine-naphthylamide, L-tryptophan-naphthylamide, L-ornithine-naphthylamide, L-glutamine-naphthylamide hydrochloride, α-L-glutamic acid-naphthylamide, glycyl-glycine-naphthylamide hydrobromide, glycyl-L-phenylalanine-naphthylamide, L-seryl-L-tyrosine-naphthylamide, L-alanyl-L-arginine-naphthylamide, L-prolyl-L-arginine-naphthylamide, glycyl-L-alanine-naphthylamide, glycyl-L-arginine-naphthylamide, L-leucyl-L-alanine-naphthylamide, L-leucyl-glycine-naphthylamide, L-phenyl-alanyl-L-arginine-naphthylamide, L-seryl-L-methionine-naphthylamide, L-histidyl-L-phenylalanine-naphthylamide, 2-naphthyl-nonanoate	+
Naphthylphosphate (pH 8.5), 2-naphthyl-caprate, L-pyrrolidonyl-naphthylamide, S-benzyl-L-cysteine-naphthylamide, L-arginyl-L-arginine-naphthylamide, L-lysyl-L-alanine-naphthylamide, L-phenylalanyl-L-prolyl-L-alanine-naphthylamide, glycyl-L-tryptophan-naphthylamide	v
L-Phenylalanyl-L-proline-naphthylamide	d
Growth in the presence of the following metal compounds or dyes:	
0.01% KSCN, 0.01% (NH$_4$)$_6$Mo$_7$O$_{24}$, 0.001% antimony sodium tartrate, 0.001% NH$_4$CuCl$_3$, 0.001% CuI$_2$, 0.01% sodium salicylate	+
0.01% KIO$_3$, 0.01% K$_2$SO$_4$, 0.01% CuO, 0.01% BaCl$_2$, 0.01% Ba(NO$_3$)$_2$, 0.01% MnCl$_2$, 0.01% MnSO$_4$, 0.01% H$_3$BO$_3$, 0.05% neutral red, 0.0001% crystal violet, 0.01% Nile blue, 0.01% Bismarck brown, 0.01%Congo red	d

(continued)

TABLE BXII.β.69. *(cont.)*

Characteristic[c]	Reaction
Growth in the presence of:	
10 μg/disk methicillin	+
10 μg/disk ampicillin, 10 μg/disk colistin sulfate, 300 U/disk polymyxin B	d

[a]For symbols see standard definitions.

[b]Data taken from Bradbury (1973, 1984), Panagopoulos (1969), Willems et al. (1987), and Van den Mooter and Swings (1990).

[c]All strains are negative for the following characteristics: oxidase; nitrate reduction; indole production; ammonia production; Voges–Proskauer test; phenylalanine deaminase; arginine dihydrolase; lysine and ornithine decarboxylase; lecithinase; hydrolysis of esculin, gelatin, casein, arbutin, sodium hippurate, and DNA; methyl red test; production of a fluorescent pigment on King's medium B; Simmons citrate; potato soft rot test; the following reactions in litmus milk: acid formation, coagulation, peptonization and reduction; β-glucosidase, lecithinase. None of the strains grew at 37°C, at pH 4.5, and 5.5, and in the presence of 30%, 20%, or 10% glucose, 2–10% NaCl, 0.1% actidione on GYEA medium (0.5% D-glucose, 0.1% yeast extract, 2% agar, trace of FePO$_4$), 0.1%, 0.05%, 0.01%, and 0.005% TTC. None of the strains produced acid from cellobiose, D-fructose, D-mannose, D-ribose, sucrose, D-xylose, salicin, *meso*-erythritol, raffinose, melibiose, trehalose, L-rhamnose, D-lactose, D-glucose, sorbitol, dulcitol, amygdalin, glycogen, inulin, dextrin, adonitol, D-mannitol, L-sorbose, α-methylglucoside, arbutin, and *meso*-inositol. None of the strains grew on D-xylose, D-mannose, D-ribose, sucrose, sorbose, lactose, raffinose, trehalose, maltose, cellobiose, L-rhamnose, D-fructose, melibiose, D-mannitol, D-sorbitol, inositol, dulcitol, adonitol, sodium-2-ketogluconate, salicin, inulin, glycogen, dextrin, arbutin, α-methylglucoside, glycerol, D-saccharic acid, mucic acid, D-glyceric acid, D-alanine, L-alanine, norleucine, L-valine, L-leucine, L-isoleucine, L-threonine, L-serine, L-asparagine, ethanolamine, *n*-butyric acid, ethanol, *iso*-butanol, formate, acetate (0.5%), calcium lactate, sodium propionate, malonate, maleate, oxalate, benzoate. All strains failed to grow on 0.5% peptone, on a medium containing NH$_4$Cl and D-glucose, and on a medium containing NH$_4$Cl, D-glucose, and mineral salts. None of the strains required L-methionine for growth or showed a stimulated growth in basal medium in the presence of L-cysteine; none used the following amino acids as sole nitrogen source: L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-tryptophan, L-valine, and L-ornithine; use the following amino acids as sole nitrogen and carbon source: L-alanine, L-hydroxyproline, L-proline, and L-asparagine. All strains failed to grow in the presence of the following metal compounds and dyes: 0.01% (NH$_4$)$_2$S$_2$O$_8$, 0.001% HgCl$_2$, 0.001% Hg(NO$_3$)$_2$, 0.001% CuCl, 0.001% CuCl$_2$, 0.001% CuSO$_4$, 0.001% Cd(CH$_3$COO)$_2$, 0.0001% Cd(CH$_3$COO)$_2$, 0.01% CoCl$_2$, 0.01% ZnCl$_2$, 0.01% Pb(CH$_3$COO)$_2$, 0.001% pyronine Y, 0.005% safranine T, 0.01% methylene blue, 0.001% malachite green, 0.005% methyl green, 0.07% methyl green, 0.005% crystal violet, 0.05% Nile blue, 0.005% diamond fuchsine, 0.01% basic fuchsin, 0.0001% brilliant green, and 0.01% thionine, and the following antibiotics (disks): 10 μg erythromycin, 10 U bacitracin, 10 μg streptomycin, 30 μg nalidixic acid, 30 μg kanamycin, 100 μg sulfafurazole, 10 U penicillin G, 25 μg cephaloridin, 10 μg gentamicin, 30 μg chloramphenicol, 10 μg fucidin, 30 μg tetracycline, 30 μg novobiocin, 30 μg neomycin, 200 μg nitrofurantoin. None of the strains hydrolyzed the following substrates in API enzymatic tests: 2-naphthyl-α-D-glucopyranoside, 2-naphthyl-β-D-galactopyranoside, 2-naphthyl-α-L-fucopyranoside, 6-Br-2-naphthyl-α-D-galactopyranoside, 6-Br-2-naphthyl-β-D-glucopyranoside, 6-Br-2-naphthyl-α-D-mannopyranoside, 1-naphthyl-*N*-acetyl-β-D-glucosaminide, naphthol-AS-BI-β-D-glucuronic acid (6-bromo-2-hydroxy-3-naphthoic acid-2-methoxyanilide-β-D-glucuronate), naphthol-AS-BI-phosphodiamide (6-bromo-2-phosphodiamide-3-naphthoic acid-2-methoxyanilide, 2-naphthyl-myristate, 2-naphthyl-laurate, 2-naphthyl-palmitate, 2-naphthyl-stearate, L-cysteine-naphthylamide, β-alanine-naphthylamide, L-histidine-naphthylamide, L-hydroxyproline-naphthylamide, L-isoleucine-naphthylamide, L-valine-naphthylamide, L-threonine-naphthylamide, γ-L-glutamic acid-naphthylamide, L-proline-naphthylamide hydrochloride, *N*-benzoyl-L-leucine-naphthylamide, *N*-benzoyl-DL-arginine-naphthylamide, *N*-benzoyl-L-alanine-4-methoxy-naphthylamide, *N*-carbobenzyloxy-L-arginine-4-methoxy-naphthylamide, *N*-glutaryl-DL-phenylalanine-naphthylamide, glycyl-L-proline-naphthylamide, L-lysyl-L-lysine-naphthylamide, α-L-aspartyl-L-alanine-naphthylamide, aspartyl-L-arginine-naphthylamide, α-L-glutamyl-α-L-glutamic acid-naphthylamide, α-L-glutamyl-L-histidine-naphthylamide, L-histidyl-L-serine-naphthylamide, *N*-acetyl-glycyl-L-lysine-naphthylamide, L-lysyl-L-alanine-4-methoxy-naphthylamide, L-alanyl-L-phenylalanyl-L-proline-naphthylamide, L-histidyl-L-leucyl-L-histidine-naphthylamide, L-valyl-L-tyrosyl-L-serine-naphthylamide, *N*-carbobenzyloxy-glycyl-glycyl-L-arginine-naphthylamide, L-alanyl-L-phenylalanyl-L-prolyl-L-alanine-naphthylamide, L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine-naphthylamide, *p*-nitrophenyl-α-D-galactopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-galactopyranoside-6-phosphate, *p*-nitrophenyl-α-L-arabinofuranoside, *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-β-D-galacturonide, *p*-nitrophenyl-β-D-glucuronide, *p*-nitrophenyl-α-maltoside, *p*-nitrophenyl-β-maltoside, *p*-nitrophenyl-*N*-acetyl-α-D-glucosaminide, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, *p*-nitrophenyl-α-L-fucopyranoside, *p*-nitrophenyl-β-D-fucopyranoside, *p*-nitrophenyl-β-L-fucopyranoside, *p*-nitrophenyl-β-D-lactoside, *p*-nitrophenyl-α-D-mannopyranoside, *p*-nitrophenyl-β-D-mannopyranoside, *p*-nitrophenyl-α-D-xylopyranoside, *p*-nitrophenyl-β-D-xylopyranoside.

[d]The following API test strips were used: API-ZYM, Osidases, Esterases, AP1, AP2, and AP3 (Biomérieux, France).

plicates isolation because fast-growing saprophytes may rapidly overgrow isolation cultures. Serfontein et al. (1997) reported the isolation of bacteria from infected material collected in hot and dry periods to be difficult. Under cool and wet conditions the same plants did yield large numbers of the bacterium. The most frequently used isolation materials are small pieces of infected wood taken aseptically from diseased vines and soaked in sterile water for 20 min. The resulting bacterial suspension is streaked onto nutrient agar (Panagopoulos, 1969; Erasmus et al., 1974; Grasso et al., 1979). Small pale-yellow colonies will appear after 5–6 d of incubation; after 8–10 d diameters of 0.4–0.6 mm are attained (Panagopoulos, 1969). An enrichment technique to improve isolation yields was proposed by Serfontein et al. (1997). Cuttings from diseased shoots that include a node were incubated in closed plastic bags together with wet cotton plugs. After 3 d in the dark at 15°C, extracts from the shoots contained significantly more *Xylophilus ampelinus* bacteria than controls analyzed before incubation. This method also permits isolation of bacteria from latently infected shoots with no apparent signs of disease (Serfontein et al., 1997). The most widely used isolation medium is nutrient agar, but better growth is obtained by adding 5% glucose to nutrient agar. YGC and GYCA medium also result in better growth, but because the $CaCO_3$ renders these media opaque they are less suitable for the study of colony morphology. YEGAL medium[3] is more convenient and also yields good growth (Starr et al., 1977). Repeated sterilization or re-melting of solid media inhibits growth of *Xylophilus* (Panagopoulos, 1969).

MAINTENANCE PROCEDURES

Cultures grown on screw-capped slants at 24°C for 2–3 d can be stored tightly closed at 4°C and should be transferred at least every 2 months. For long-term preservation, strains can be lyophilized.

DIFFERENTIATION OF THE GENUS *XYLOPHILUS* FROM OTHER GENERA

The most typical feature of *Xylophilus* strains is their extremely slow and poor growth on most media at the optimal growth temperature of 24°C. They can be differentiated from most *Xanthomonas* species by the following characteristics: very low salt tolerance, inability to produce acid from numerous sugars, failure to hydrolyze gelatin and esculin, positive urease reaction, growth on K,Na-tartrate, L-glutamic acid, absence of growth on sucrose, and the use of L-glutamic acid as a sole carbon and nitrogen source (Van den Mooter and Swings, 1990). Other slow-growing organisms can also be isolated from grapevines but many of these are Gram positive. Gram reaction, catalase and oxidase tests, urease production, and lipolysis of Tween 80 have been proposed as preliminary confirmation tests to distinguish *Xylophilus ampelinus* from other slow growers (Serfontein et al., 1997).

Serological tests with specific antisera are used for rapid identification of the pathogen (Erasmus et al., 1974; Ridé, 1996).

TAXONOMIC COMMENTS

Xylophilus ampelinus was first classified in the genus *Xanthomonas* because it is a Gram-negative, aerobic, nonsporeforming plant pathogen with monotrichously flagellated rod-shaped cells, produces a yellow water-insoluble pigment and metabolizes sugars oxidatively (Panagopoulos, 1969). Later it became evident from additional data, such as pigment analyses (Starr et al., 1977), comparative studies of the biosynthesis of aromatic amino acids (Whitaker et al., 1981; Byng et al., 1983) and DNA–rRNA hybridization studies comparing *Xanthomonas ampelina* DNA with *Xanthomonas campestris* (De Vos and De Ley, 1983) that the vine pathogen was not a genuine *Xanthomonas*. As a result of a study of 34 strains from different geographic origins by means of SDS-polyacrylamide gel electrophoresis of whole-cell proteins, numerical analysis of 106 enzymatic features, and DNA–DNA and DNA–rRNA hybridization, we created a separate genus *Xylophilus* for these organisms (Willems et al., 1987). DNA–rRNA hybridization demonstrated that *Xylophilus* is a member of the family *Comamonadaceae* (previously called the "acidovorans rRNA complex"). Using this method none of the other diverse organisms of this family seemed especially closely related to the vine pathogen. Some phytopathogenic *Pseudomonas* species (now *Acidovorax*) and *Alcaligenes paradoxus* strains yielded slightly more stable DNA–rRNA hybrids when their DNA was hybridized with labeled rRNA of *X. ampelinus*, but when taking into account the experimental error, this was not considered significant (Willems et al., 1987). More recently the 16S rDNA sequences of *Xylophilus ampelinus* and various other members of the *Comamonadaceae* were determined, and these data revealed a closer relationship between *Xylophilus ampelinus* and *Variovorax paradoxus*. A sequence similarity of 97.9% was reported between both type strains (Wen et al., 1999). In other genera of the *Comamonadaceae* (*Acidovorax*, *Comamonas*, and *Hydrogenophaga*), this level of similarity is found between species within a genus. Together with the absence of DNA binding between the type strains of *Xylophilus* and *Variovorax* (Willems et al., 1991a), these would be arguments in support of a merger of these genera. Both are yellow-pigmented, although it is unknown whether these pigments have a similar structure. On the other hand, *Xylophilus* grows extremely slowly and is a phytopathogen with a rather restricted metabolic versatility, whereas *Variovorax* uses a wide variety of substrates and some of its strains are capable of chemolithotrophic growth using hydrogen oxidation as an energy source. In view of these differences and until further genotypic similarities are reported, the maintenance of separate genera may be justified for these organisms.

FURTHER READING

Ridé, M. 1996. La nécrose bactérienne de la vigne: données biologiques et épidémiologiques, base d'une stratégie de lutte. Comptes rendus de l'Académie d'Agriculture de France. *82*: 31–50.

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3. YEGAL medium (g /l distilled water): yeast extract, 5.0; galactose, 10.0; K_2HPO_4, 4.01; NaH_2PO_4, 4.55; NH_4Cl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; ferric ammonium citrate, 0.05; $CaCl_2$, 0.005. Yeast extract and galactose are each dissolved in 100 ml of water and autoclaved separately and added aseptically (Starr et al., 1977).

List of species of the genus Xylophilus

1. **Xylophilus ampelinus** (Panagopoulos 1969) Willems, Gillis, Kersters, Van Den Broecke, and De Ley 1987, 428[VP] (*Xanthomonas ampelina* Panagopoulos 1969, 75.)

am.pe.li′nus. Gr. n. *ampelos* grape vine; Gr. adj. *ampelinos* M.L. masc. adj. *ampelinus* of the vine.

The morphological and cellular characteristics are as

described for the genus. Additional descriptive information is presented in Table BXII.β.69, which is based on data from Panagopoulos (1969), Bradbury (1973, 1984), Willems et al. (1987), and Van den Mooter and Swings (1990).

Isolated from *Vitis vinifera*, where it causes bacterial necrosis and canker, in the Mediterranean area and South Africa. Similar symptoms on grape vine have been reported from Argentina, Austria, Bulgaria, the Canary Islands, and Switzerland (Bradbury, 1973).

The mol% G + C of the DNA is: 68–69 (T_m).

Type strain: ATCC 33914, DSM 7250, LMG 5856, NCPPB 2217.

GenBank accession number (16S rRNA): AF078758.

Order II. **Hydrogenophilales** *ord. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Hy.dro.ge.no.phi.la' les. M.L. masc. n. *Hydrogenophilus* type genus of the order; *-ales* ending to denote order; M.L. fem. n. *Hydrogenophilales* the *Hydrogenophilus* order.

The order *Hydrogenophilales* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA gene sequences; the order contains the family *Hydrogenophilaceae*.

Description is the same as for the family *Hydrogenophilaceae*.

Type genus: **Hydrogenophilus** Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785.

Family I. **Hydrogenophilaceae** *fam. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Hy.dro.ge.no.phi.la' ce.ae. M.L. masc. n. *Hydrogenophilus* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Hydrogenophilaceae* the *Hydrogenophilus* family.

The family *Hydrogenophilaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA gene sequences; the family contains the genera *Hydrogenophilus* (type genus) and *Thiobacillus*.

Obligate or facultative chemolithotrophs, using H_2 (*Hydrogenophilus*) or reduced sulfur compounds (*Thiobacillus*) as electron donors. Fix carbon by the Calvin-Benson cycle.

Type genus: **Hydrogenophilus** Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785.

Genus I. **Hydrogenophilus** Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785[VP]

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Hy.dro.ge.no' phi.lus. Gr. n. *hydro* water; Gr. v. *genein* to produce; M.L. neut. n. *hydrogenum* hydrogen (that which produces water); Gr. adj. *philo* loving, friendly to; M.L. masc. n. *Hydrogenophilus* hydrogen lover.

Straight rods 0.5–0.8 × 1.0–3.0 μm during exponential growth. Occur singly. Motile or nonmotile. Gram negative. Nonsporulating. Aerobic or microaerophilic, having a strictly respiratory type of metabolism, with oxygen as the terminal electron acceptor. **Colonies are yellow. Thermophilic**; one species grows optimally at 50–52°C; and a second, at 60–65°C. **Facultatively chemolithoautotrophic**; can use H_2 as an electron donor and CO_2 as a carbon source. CO_2 is fixed via the Calvin-Benson cycle. Carbohydrates are not utilized. Acetate, pyruvate, DL-lactate, succinate, and DL-malate can be used as electron donors and carbon sources. Ammonium can be used as a nitrogen source. The major quinone system is ubiquinone 8. Isolated from hot springs and surrounding soil.

The mol% G + C of the DNA is: 61–65.

Type species: **Hydrogenophilus thermoluteolus** Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785.

FURTHER DESCRIPTIVE INFORMATION

Hayashi et al. (1999) found the type strain (TH-1) of *H. thermoluteolus* to be nonmotile; however, Goto et al. (1978) reported the same strain to be motile. Reference strain TH-4 is nonmotile (Hayashi et al., 1999). The type strain of *H. hirschii* is motile by a single polar flagellum.

ENRICHMENT AND ISOLATION PROCEDURES

Isolation and culture conditions for *H. thermoluteolus* have been described by Goto et al. (1977). The isolation of *H. hirschii*—a microaerophilic organism—has been described by Stöhr et al. (2001); briefly, the autotrophic medium of Huber et al. (1992) was used lacking NaCl but containing 0.02% $CaCl_2 \cdot 2H_2O$. The medium was deoxygenated with N_2 and dispensed in 20-ml portions into 120 ml serum bottles under N_2. The gas phase in the bottles was changed to H_2/CO_2 (80:20, v/v), and the medium was sterilized at 100°C for 90 min and cooled. To each bottle was added 100 μl of a 10% sterile solution of $CaCO_3$ and 20 ml of filter-sterilized air. After several passages of serially diluted cultures, the cultures were plated onto the isolation medium solidified with 1.5% agar to obtain yellow colonies consisting of motile rods.

DIFFERENTIATION OF THE GENUS *HYDROGENOPHILUS* FROM OTHER GENERA

The genus *Hydrogenophilus* differs from the genera *Calderobacterium* and *Hydrogenobacter* in that it consists of facultative hydrogen autotrophs instead of obligate hydrogen autotrophs. Moreover, the optimal growth temperatures of *H. thermoluteolus* and *H. hirschii* (50–52°C and 63°C, respectively) are lower than that of *Calderobacterium* (74–76°C; Kryukov et al., 1983) and of *Hydrogenobacter* (70–75°C; Kawasumi et al., 1984). In addition, the cell diameter of *H. thermoluteolus* and *H. hirschii* (0.5–0.6 μm and 0.6–0.8 μm, respectively) is greater than that of *Calderobacterium* (0.25–0.5 μm) and *Hydrogenobacter* (0.3–0.5 μm) cells.

TAXONOMIC COMMENTS

Based on DNA–DNA hybridization experiments, Hayashi et al. (1999) found strain TH-1—the type strain of *H. thermoluteolus*—and reference strain TH-4 to have a hybridization value of 89%,

indicating that the two strains belong to the same species. Moreover, 16S rDNA analysis indicated that, although the two strains belonged to the class *Betaproteobacteria*, they were sufficiently unrelated to this class as to warrant their placement in a new genus, *Hydrogenophilus* (Hayashi, et al., 1999). Analysis of 16S rDNA sequences by Stöhr et al. (2001) indicated that strain TH-1—the type strain of *H. thermoluteolus*—has a phylogenetic distance of 0.0257 from the type strain of *H. hirschii* (Yel5a); thus the two type strains should be placed within the same genus.

In this edition of the *Manual*, the genus *Hydrogenophilus* is classified in the Order *Hydrogenophilales* and the Family *Hydrogenophilaceae* within the Class *Betaproteobacteria*. The only other member of the family is the genus *Thiobacillus*, which differs markedly in its phenotypic properties from *Hydrogenophilus*. Other genera of Gram-negative, thermophilic, hydrogen chemolithotrophs include *Calderobacterium* and *Hydrogenobacter*, which are classified in the Phylum *Aquificae*, Class *Aquificae* Order *Aquificales*, and Family *Aquificaceae*.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *HYDROGENOPHILUS*

Hydrogenophilus thermoluteolus grows optimally at 50–52°C, whereas *H. hirschii* grows best at 63°C. In regard to their relationship to oxygen, *H. thermoluteolus* grows best at 22% O_2, whereas *H. hirschii*

is a microaerophile that grows best at 2.5% O_2 and fails to grow at O_2 levels higher than 5%.

List of species of the genus Hydrogenophilus

1. **Hydrogenophilus thermoluteolus** Hayashi, Ishida, Yokota, Kodama and Igarashi 1999, 785[VP]

   ther.mo.lu.te′ o.lus. Gr. adj. *thermos* hot; L. adj. n. *luteolus* light yellow; M.L. masc. adj. *thermoluteolus* hot and light yellow.

   Exponentially growing cells are 0.5–0.6 × 2.0–3.0 μm. Optimal temperature, 50–52°C. Optimal pH, 7.0. Aerobic. Other characteristics are as given for the genus, with the following additional information. Carbon sources include propionate, butyrate, and α-ketoglutaric acid. No growth occurs on lactose, D-glucose, D-galactose, sucrose, citrate, ethanol, benzoate, and *m*-hydroxybenzoate. Ammonium, nitrate, and urea can be used as sole nitrogen sources; nitrite and N_2 are not used. Major fatty acids: $C_{16:0}$, $C_{18:0}$. The major 3–hydroxy cellular fatty acid is $C_{10:0\ 3OH}$. Cyclic fatty acids have not been described in *H. thermoluteolus*. Isolated from soil around a hot spring in Izu peninsula, Shizuoka Prefecture, Japan (Goto et al., 1977).

   The mol% G + C of the DNA is: 63–65 (T_m).
   Type strain: TH-1, IFO 14978.
   GenBank accession number (16S rRNA): AB009828.

2. **Hydrogenophilus hirschii** Stöhr, Waberski, Liesack, Völker, Wehmeyer and Thomm 2001, 488[VP]

   hir′ schi.i. N.L. gen. n. *hirschii* in honor of Peter Hirsch, in recognition of his fundamental contributions to the taxonomy of unusual bacteria.

   Cells are 0.6–0.8 × 1.0–2.0 μm. Motile by a single polar flagellum. Optimal growth temperature, 63°C; upper limit, 68°C. No growth at 45°C. Microaerophilic, growing best at 2.5% O_2 and failing to grow at O_2 levels higher than 5%. Other characteristics are as given for the genus, with the following additional information. Growth occurs anaerobically with nitrate. Growth occurs on yeast extract, peptone, meat peptone, and meat extract. Fumarate, glutamate, and gluconate can be used as carbon sources. No growth occurs on carbohydrates, aromatic compounds, L-alanine, L-proline, citric acid, methanol, or ethanol. Neither thiosulfate nor sulfur is used as an electron donor. Major fatty acids: $C_{16:0}$, $C_{17:0\ cyclo}$, and $C_{19:0\ cyclo}$. Isolated from a water sample from Angel Terrace in Yellowstone National Park, U.S.A.

   The mol% G + C of the DNA is: 61 (HPLC).
   Type strain: Yel5a, DSM 11420, JCM 10831.
   GenBank accession number (16S rRNA): AJ131694.

Genus II. **Thiobacillus** Beijerinck 1904b, 597[AL]

DONOVAN P. KELLY, ANN P. WOOD AND ERKO STACKEBRANDT

Thi.o.ba.cil′ lus. Gr. n. *thios* sulfur; L n *bacillus* a small rod; M. L. masc n. *Thiobacillus* sulfur rodlet.

Small, Gram-negative, rod-shaped cells (0.3–0.5 × 0.9–4.0 μm). Some species are motile by means of polar flagella. No resting stages known. Energy is derived by the oxidation of one or more reduced sulfur compounds, including sulfides, sulfur, thiosulfate, polythionates, and thiocyanate. Sulfate is the end product of sulfur-compound oxidation, but sulfur, sulfite, and polythionates may be accumulated by most species, sometimes transiently. All

species can fix carbon dioxide by means of the Benson–Calvin cycle and are capable of autotrophic growth; some species are obligately chemolithotrophic, while others are chemoorganotrophic. The genus currently includes obligate aerobes and facultative denitrifiers. Optimal pH of 2–8 with optimal temperature of 28–43°C. Distribution is seemingly ubiquitous in marine, freshwater, and soil environments, especially where oxidizable sulfur

is abundant (e.g., sulfur springs, sulfide minerals, sulfur deposits, sewage treatment areas, and sources of sulfur gases, such as sediments or anaerobic soils releasing H_2S).

The mol% G + C of the DNA is: 62–67.

Type species: **Thiobacillus thioparus** Beijerinck 1904b, 597.

FURTHER DESCRIPTIVE INFORMATION

The chemolithotrophic, sulfur-compound-oxidizing, Gram-negative, rod-shaped members of the genera and species described (Table BXII.β.70) are usually 0.5 × 1.0–4.0 µm in size, occurring singly, in pairs or in short chains; some are motile by means of single polar flagella; some possess pili and other specialized surface features. All can obtain energy from the oxidation of reduced inorganic sulfur compounds, and, in most cases, elemental sulfur. Some species are obligately chemolithotrophic and autotrophic, others are facultatively heterotrophic, and, in some strains, optimal growth occurs mixotrophically. Both electron-transport-dependent and substrate-level phosphorylation occur during sulfur-compound oxidation, and reduction of NAD(P) requires an energy-dependent flow of electrons from cytochrome c (or b). Carbon dioxide fixation occurs mainly by means of the Benson–Calvin cycle, with some fixation occurring through pyruvate or phosphoenolpyruvate carboxylation. The obligately autotrophic species possess an incomplete tricarboxylic acid cycle (lacking 2-oxoglutarate dehydrogenase), which is used as a biosynthetic "horseshoe" pathway via oxaloacetate or succinate and 2-oxoglutarate, respectively, down each arm of the "horseshoe". Some species contain characteristic plasmids, and some have been shown to be susceptible to introduction of Pseudomonas plasmids. Little work on the genetics of Thiobacillus spp. is available, but it is known that autotrophic and drug-resistant mutants can be obtained and that mutations that decrease autotrophic efficiency can be induced. No species is known to be pathogenic. Thiobacillus spp. are ubiquitous, with the facultative species occurring in soil, freshwater, and marine environments as heterotrophs or mixotrophs. Sulfur-compound-oxidizing species have been isolated from Arctic, temperate, and tropical waters, soil, salt marshes, freshwater lakes, rivers, canals, hot springs, sulfur-rich mine or acid-mine wastewaters and other environments where oxidizable sulfur compounds occur naturally or anthropogenically.

ENRICHMENT AND ISOLATION PROCEDURES

Most of the species can be isolated from natural habitats by the use of mineral media containing elemental sulfur or thiosulfate as the energy-yielding substrate. Use of media of different pH will assist in differential selection of the neutrophilic and acidophilic species, use of acid ferrous sulfate medium will frequently select for Acidithiobacillus ferrooxidans, and use of anaerobic thiosulfate medium (pH 7) supplemented with nitrate will select for T. denitrificans. A procedure has been described for the enrichment of facultatively autotrophic, mixotrophic strains, using a continuous-flow chemostat with both organic and inorganic substrates (Gottschal and Kuenen, 1980). This provides a means of avoiding the predomination by heterotrophs in standard batch enrichment media containing supplements such as thiosulfate and glucose or thiosulfate and acetate. In the latter medium, a mixture of obligately chemolithotrophic thiobacilli and chemoorganotrophs normally develops. Acidiphilium acidophilum was originally isolated as a commensal of A. ferrooxidans, although "T. organoparus" (now considered to be a strain of A. acidophilum) was enriched directly from an acid mine water environment. Media suitable for the different species are summarized in Kelly and Wood (1998) and are available from the original literature describing the species. In addition, culture collection catalogues also provide guidance for media suitable for their cultures.

Most strains are able to produce colonial growth on appropriate media solidified with agar. Some strains, especially those of A. ferrooxidans, grow poorly on agar media. In some cases, this difficulty is due to the toxicity of agar hydrolysis products and has been overcome in a number of ways (e.g., Tuovinen and Kelly, 1973). Toxic effects are generally avoided by the use of a minimal concentration of agar, screening of suitable brands of purified agars, use of agarose, and, in the case of A. ferrooxidans, use of a combination of media with low agar concentrations, at

TABLE BXII.β.70. Differentiation of the genera of chemolithotrophic, sulfur-oxidizing, rod-shaped bacteria

Character	Thiobacillus	Acidiphilium	Acidithiobacillus	Halothiobacillus	Paracoccus	Starkeya	Thermithiobacillus	Thiomonas
Obligate chemolithoautotroph[a]	+	−	+	+	−	−	+	−
Heterotrophic growth on defined media	−	+[b]	−	−	+[c]	+	−	−[d]
Mol% G + C of DNA	62–67	63–68	52–64	56–67	63–71	67–68	66–67	61–67
Class:								
Alphaproteobacteria		+			+	+		
Betaproteobacteria	+							+
Gammaproteobacteria			+	+			+	
Respiratory ubiquinone	Q-8	Q-10	Q-8	Q-8	Q-10	Q-10	Q-8	Q-8
Facultative denitrification	+[e]	−	−	−	+[c]	−	−	−
Optimal temperature (°C)	28–43	25–37	30–45	28–30	25–37	25–30	43–45	30–50
Optimal pH	6.8–8.0[f]	3.0–3.5	2.0–3.5	6.5-8.0	6.5–9.0	7	6.8–7.5	5.2–6.0[g]
Halophilic or halotolerant	−	−	−	+	−	−	−	−
Contains photosynthetic reaction centers	−	+	−	−	−	−	−	−

[a]With inorganic sulfur compounds as sole energy substrates.

[b]Most species of the genus are not chemolithoautotrophic.

[c]Not all species exhibit all these features, and only P. denitrificans , P. versutus, and P. pantotrophus are facultative, sulfur-oxidizing chemolithoautotrophs.

[d]Most strains will grow on complex rich media, and some grow best mixotrophically with thiosulfate plus organic supplements.

[e]Denitrification to dinitrogen in one species only: T. denitrificans.

[f]T. plumbophilus grows only between pH 4.0–6.5.

[g]Optimum for T. cuprinus is pH 3.0–4.0.

pH 2.2–2.5, and with ferrous sulfate at only about 20 mM. The use of silica gel media as an alternative is normally unnecessary.

Maintenance Procedures

After cultivation on suitable media, most species survive storage at 5°C for periods of weeks to months, especially if the media for neutrophiles have not become too acidic before storage. *A. ferrooxidans* survives in culture on pyrite for very long periods when stored at 5 to 15°C, and *"T. plumbophilus"* survives for at least a year at room temperature on galena (Drobner et al., 1992). Many strains have been successfully lyophilized or have survived storage at −20°C, at liquid nitrogen temperature, or in glycerol suspension at −20°C.

Differentiation of the genus *Thiobacillus* from other genera

At the time of the previous edition of this *Systematics*, information on ubiquinone and fatty acid content, DNA base composition, and interspecific DNA–DNA hybridization had been obtained for the thiobacilli (Kelly and Harrison, 1989). This enabled division of the species into distinct groups on the basis of physiological and biochemical characteristics (Harrison, 1982, 1983; Katayama-Fujimura et al., 1982; Katayama-Fujimura and Kuraishi, 1983). It has also complicated consolidation of species, as DNA–DNA hybridization studies in some cases show considerable diversity among strains regarded as members of the same species, while also showing very high levels of similarity between putatively different species. The confirmation by 16S rRNA sequencing of the phylogenetic diversity of the formally approved species now forces major revision of this genus.

Primary separation of most of the former 21 species of *Thiobacillus* is relatively easy to achieve by virtue of differences in gross physiological characteristics, such as pH and temperature requirements for growth, ability or inability to grow heterotrophically as well as chemolithotrophically, and differences in the ability to grow anaerobically with denitrification or to use elemental sulfur. Most species designated as *Thiobacillus* may precipitate elemental sulfur into the medium during growth on sulfide, thiosulfate, and, in some cases, trithionate or tetrathionate. One of the exceptions is *T. versutus*. Sulfur precipitation is not a highly distinctive diagnostic characteristic, as it is a variable property influenced by growth conditions, such as oxygen availability or perturbation of steady state in chemostat culture. The precipitation of sulfur is superficially comparable to the extracellular precipitation observed with species of *Chlorobium* and contrasts with the intracellular accumulation of sulfur by members of the *Chromatiaceae* such as *Chromatium*. It is likely, however, that some species deposit sulfur internally, subsequently oxidizing it to sulfate. Thus, *T. albertensis* grown on thiosulfate is believed to contain a sulfur granule bounded by a membrane (Bryant et al., 1983), the appearance of which is correlated with the production of a large amount of extracellular elemental sulfur at the end of the growth phase. Morphologically, these inclusions resemble the sulfur found in *Beggiatoa* and *Thiothrix*. These granules do not appear during mid-log growth (Bryant et al., 1983), and had not been reported in earlier ultrastructural surveys of other thiobacilli (Mahoney and Edwards, 1966; Shively et al., 1970), but may have been observed in *"T. kabobis"* (Reynolds et al., 1981), which is now regarded as a synonym of *T. thiooxidans* (Kuenen et al., 1992). The possibility of both extracellular deposition and intracellular accumulation of elemental sulfur in some thiobacilli is somewhat of a physiological anomaly,

possibly indicative of differing mechanisms or locations of sulfur compound oxidation. Extracellular sulfur precipitation is best explained as a consequence of the conversion of sulfide or the sulfane groups of thiosulfate (or polythionates) to sulfur at the surface of the cell, presumably in the periplasmic space and catalyzed by enzyme systems located in or external to the bounding membrane of the cell. This seems to be more plausible than intracellular generation of sulfur and its excretion to the outside (as has indeed been suggested). The physicochemical nature of the sulfur at the moment of formation is uncertain, and, of course, the cell wall would likely be a barrier to the excretion of large granules from inside the cell or even to their generation in the periplasmic space. The production of intracellular sulfur from a soluble substrate (thiosulfate) implies intracellular oxidation of that substrate and transport of S^0 across the membrane. Although it is clear that this process would involve membrane-associated electron transport systems in the oxidation of sulfur compounds to sulfate, the mechanism remains to be elucidated. Clearly, the present stage of our knowledge of the mechanism of transport of sulfur and its compounds and of the biochemistry of their conversion to sulfate is inadequate. Therefore, characteristics such as intracellular sulfur accumulation cannot be employed as reliable taxonomic features.

Taxonomic Comments

Since the description of *Thiobacillus* was published in the first edition of the *Systematics* (Kelly and Harrison, 1989), the genus has undergone a number of revisions and emendations. These changes are summarized in Table BXII.β.70 and Table BXII.β.71. Table BXII.β.71 also presents the DNA base composition and class within the *Proteobacteria* to which each species is now assigned, based on sequence analysis of either partial or complete 16S rRNA genes and recently proposed reassignments to new genera (Kelly and Wood, 2000a, b; Kelly et al., 2000). A phylogenetic tree illustrating the new and different genera to which most of the *Thiobacillus* species have been assigned is shown in Fig. BXII.β.60.

First, *T. versutus* and *T. acidophilus* (both members of the class *Alphaproteobacteria*) have been formally transferred from *Thiobacillus* into other genera (Katayama et al., 1995; Hiraishi et al., 1998; see the chapter by Hiraishi and Imhoff on the genus *Acidiphilium* in the family *Acetobacteraceae* in this volume), and each appears elsewhere within this edition. The remaining species group within the classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. The type species, *T. thioparus*, falls in the *Betaproteobacteria*, along with the closely related *T. denitrificans* and the moderate thermophile *T. aquaesulis* (McDonald et al., 1997; Kelly and Wood, 2000a, b). These three species are the only ones that can be justified based on molecular criteria to be members of the genus *Thiobacillus*. A fourth validly named species, *Thiobacillus delicatus*, is physiologically similar to members of the genus *Thiomonas* and may be reassigned to that genus when more data are available (Y. Katayama, personal communication). *Thiobacillus denitrificans* has been regarded as a close relative of *T. thioparus* since it was originally isolated by Beijerinck (1904a, b), and that view is now strongly supported by the confirmation that it is a member of the *Betaproteobacteria* (Lane et al., 1992; H.G. Trüper, personal communication, 1999; Kelly and Wood, 2000a). Of the remaining species, only *Thiobacillus novellus* has been shown to be a member of the *Alphaproteobacteria*. *T. novellus* is facultatively autotrophic (as are the *Alphaproteobacteria* species formerly classified as *T. versutus* (now *Paracoccus versutus*) and *T.*

TABLE BXII.β.71. Species of *Thiobacillus* recognized in the first edition of this *Manual* (Kelly and Harrison, 1989), or described subsequently, showing those species assigned, or proposed for assignment, to new or different genera

Basonym	Revised genus designations (published or proposed)
Thiobacillus thioparus [a]	*Thiobacillus thioparus*[T]
Thiobacillus denitrificans[b]	*Thiobacillus denitrificans*
Thiobacillus aquaesulis[c]	*Thiobacillus aquaesulis*
Thiobacillus plumbophilus[d]	Uncertain (currently *Thiobacillus*)
Thiobacillus intermedius[e]	*Thiomonas intermedia*[T]
Thiobacillus perometabolis[e]	*Thiomonas perometabolis*
Thiobacillus cuprinus[e]	*Thiomonas cuprina*
Thiobacillus thermosulfatus[e]	*Thiomonas thermosulfata*
Thiobacillus delicatus[f]	*Thiomonas delicata*
Thiobacillus neapolitanus[g]	*Halothiobacillus neapolitanus*[T]
Thiobacillus halophilus[g]	*Halothiobacillus halophilus*
Thiobacillus hydrothermalis[g]	*Halothiobacillus hydrothermalis*
Thiobacillus tepidarius[g]	*Thermithiobacillus tepidarius*[T]
Thiobacillus thiooxidans[g]	*Acidithiobacillus thiooxidans*[T]
Thiobacillus ferrooxidans[g]	*Acidithiobacillus ferrooxidans*
Thiobacillus caldus[g]	*Acidithiobacillus caldus*
Thiobacillus albertensis[d]	(*Acidithiobacillus*)
Thiobacillus prosperus[d]	Uncertain ("*Acidihalobacter*")
Thiobacillus novellus[h]	*Starkeya novella*
Thiobacillus acidophilus[i]	*Acidiphilium acidophilum*
Thiobacillus versutus[j]	*Paracoccus versutus*

[a]Kelly and Harrison, 1989.

[b]Kelly and Wood, 2000a; see text.

[c]McDonald et al., 1997.

[d]See text.

[e]Moreira and Amils, 1997.

[f]Requires investigation.

[g]Kelly and Wood, 2000b.

[h]Kelly et al., 2000.

[i]Hiraishi et al., 1998.

[j]Katayama et al., 1995.

FIGURE BXII.β.60. Phylogenetic tree based on 16S rRNA gene sequences of representatives of the genera to which species formerly described as *Thiobacillus* are now assigned (*Thiobacillus, Acidiphilium, Starkeya, Thermithiobacillus, Acidithiobacillus, Halothiobacillus,* and *Thiomonas*). Reference species are taken from the Ribosomal Database Project (RDP) or from the sequence database libraries. *T* indicates the type strain of the species. Bar = 10 inferred nucleotide changes per 100 nucleotides. Numbers at some nodes indicate the number of times the species to the right occurred in 100 bootstrap replicates.

acidophilus (now *Acidiphilium acidophilum*), as well as *Thiosphaera pantotropha* (now *Paracoccus pantotrophus*) and has been proposed to be the type species of a new genus, *Starkeya* (Kelly et al., 2000). Of the other species in the *Betaproteobacteria*, four have been reassigned to the new genus *Thiomonas* (Moreira and Amils, 1997). A fifth acidophilic, lead-sulfide-leaching species reportedly also in the *Betaproteobacteria*, "*Thiobacillus plumbophilus*" (Drobner et al., 1992), has been too little studied to know if it should also be reassigned to *Thiomonas*, so for the present time it is retained in the genus *Thiobacillus*. Of the species within the *Gammaproteobacteria*, a broad division can be seen between two principal groups: one comprising the proposed new genus *Halothiobacillus* (McDonald et al., 1997; Kelly et al., 1998a; Kelly and Wood, 2000b), and the other (*Acidithiobacillus*) containing at least three of the acidophilic species, some of which also oxidize Fe^{2+} and sulfide minerals (Table BXII.β.71; Fig. BXII.β.60). The former *Thiobacillus tepidarius* also seems to be somewhat distantly related to this group (McDonald et al., 1997; Goebel et al., 2000), but we consider its moderate thermophily and lack of acidophily sufficient grounds to regard it as a distinct genus (*Thermithiobacillus*) at this time (Kelly and Wood, 2000b). *Thiobacillus albertis* (whose specific epithet is here corrected to *albertensis*) is also acidophilic and strictly chemolithotrophic, making it physiologically similar to *Acidithiobacillus thiooxidans*, but it differs from that species in possessing a glycocalyx and having a mol% G + C of 61.5, which is significantly higher than that of *A. thiooxidans* (50–

52 mol% G + C). Based on 16S rRNA sequences, we confirm the placement of *T. albertensis* in *Acidithiobacillus* (Table BXII.β.71), whose members have DNA mol% G + C values ranging between 50–64 (Table BXII.β.71). "*T. prosperus*" is more difficult to define taxonomically, as it appears to be completely unrelated to the *T. ferrooxidans–T. thiooxidans–T. caldus* cluster based on 16S rRNA sequence comparisons (Goebel et al., 2000) and shows negligible DNA–DNA hybridization with other thiobacilli. We have reported the acidophilic species *T. ferrooxidans, T. thiooxidans,* and *T. caldus,* as well as *T. tepidarius,* to fall in the *Gammaproteobacteria*, whereas some reports in the literature assign these to the *Betaproteobacteria*. It seems clear to us from recent studies that these species, while close to the beta–gamma separation, are rightly placed in the *Gammaproteobacteria* (McDonald et al., 1997; Goebel et al., 2000; Kelly and Wood, 2000b).

Descriptions of the former *Thiobacillus* species *T. versutus* and *T. acidophilus* are found in the chapters on *Paracoccus* and *Acidiphilium*, respectively. Some characteristics of the genus *Thiobacillus* of the newly created genera, and of *Paracoccus* and *Acidiphilium* are given in Table BXII.β.70.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *THIOBACILLUS*

The characteristics of each of the six species are summarized in Table BXII.β.72.

List of species of the genus Thiobacillus

1. **Thiobacillus thioparus** Beijerinck 1904b, 597[AL]

   thi.o′par.us. G. n. *thios* sulfur; M.L. adj. *thioparus* sulfur producing.

   Rods, averaging 0.5 × 1.7 μm. Motile with a polar flagellum. Gram negative. Colonies grown on thiosulfate agar (1–2 mm in diameter) are circular and whitish-yellow due to precipitated sulfur. Turn pink, then brown on aging, especially in the center of old colonies. In static culture in liquid thiosulfate medium, sulfur is precipitated, and the medium becomes turbid with a pellicle of sulfur and cells. Sulfur granules and tetrathionate and/or trithionate may accumulate, accompanied by a pH drop to 4.5. In well-aerated or chemostat culture, sulfur oxidation of thiosulfate to sulfate may occur without precipitation. Some strains oxidize thiocyanate, thiosulfate, trithionate, tetrathionate, sulfur, and sulfide. Obligately chemolithotrophic and autotrophic. Ammonium salts and nitrates used as nitrogen sources. Aerobic. Optimal temperature: 28°C. Optimal pH: 6.6–7.2, with growth occurring between pH 4.5 and 7.8. Some strains are claimed to grow at pH 10.0. Found in mud, soil, canal water, and other freshwater sources. Presumably widely distributed.

   The mol% G + C of the DNA is: 62–63 (Bd, T_m).
   Type strain: ATCC 8158, DSM 505.
   GenBank accession number (16S rRNA): M79426.

2. **Thiobacillus aquaesulis** Wood and Kelly 1995, 418[VP] (Effective publication: Wood and Kelly 1988, 342.)

   a.quae.su′lis. L. n. *aquae* waters; L. n. *Sulis* pertaining to the Temple of Sulis Minerva (Minerva, the Roman goddess of wisdom); M.L. adj. *aquaesulis* from the waters of Sulis Minerva.

   Short rods, 0.3 × 0.9 μm, containing some polyphosphate inclusions. Motile. Gram negative. Non-sporeforming. Chemolithoautotrophic growth on thiosulfate, trithionate, or tetrathionate. Colonies on thiosulfate agar at 43°C are small (1–2 mm), circular, convex, and smooth, becoming white or yellow with precipitated sulfur. In liquid batch culture, sulfur precipitation and a drop in pH without tetrathionate accumulation occur. Initiates growth at pH 7–9 (30–55°C), dropping the pH to 6–7. No growth at pH 6.4 or 9.4 or at 26°C or 58°C. Chemostat cultures do not accumulate sulfur or other intermediates during growth on thiosulfate, trithionate, or tetrathionate at pH 7.6 and 43°C. Facultatively heterotrophic on complex media (yeast extract or nutrient broth) but unable to grow on common sugars, organic acids, formate, or methylamine as single substrates. Uses ammonium salts as nitrogen sources. Capable of anaerobic, autotrophic growth during thiosulfate-dependent denitrification in batch culture, producing nitrite and sulfur from thiosulfate and nitrate. Optimal temperature: 40–50°C. Optimal pH: 7.5–8.0. Contains ubiquinone-8. Isolated from the thermal springs at Bath, Avon, England.

   The mol% G + C of the DNA is: 65.7 (T_m).
   Type strain: ATCC 43788, DSM 4255.
   GenBank accession number (16S rRNA): U58019.

3. **Thiobacillus delicatus** (ex Mizoguchi, Sato and Okabe 1976)[VP] Katayama-Fujimura, Kawashima, Tsuzaki and Kuraishi 1984a, 142.

   del.i.cat′us. L. masc. adj. *delicatus* delicate.

   Rods, usually single, rarely in pairs, 0.4–0.6 × 0.7–1.6 μm. Nonmotile. Colonies grown on yeast extract–thiosulfate agar (1 mm. diameter) are smooth and circular and

TABLE BXII.β.72. Basic characteristics of species of the genus *Thiobacillus*

Characteristic	*T. thioparus*	*T. aquaesulis*	*T. denitrificans*	"*T. plumbophilus*"	*T. delicatus*	"*T. prosperus*"
Mol% G + C	62–63	66	63	66	66–67	64
Cell size (μm)	0.5 × 1.7	0.3 × 0.9	0.5 × 1.0–3.0	0.25 × 3.0	0.4–0.6 × 0.7–1.6	0.3 × 3–4
Motility	+	+	+	+	−	+
Carboxysomes	+	nd	−	nd	nd	+
Obligately chemolithoautotrophic	+	−	+	+	−	+
Optimal pH	6.6–7.2	7.5–8.0	6.8–7.4	4.0–6.5	5.5–6.0	nd
pH limits	4.5–7.8	6.5–9.0	4.5–8.0	4.0–6.5	5.0–7.0	1.0–4.5
Optimal temperature (°C)	28	40–50	28–32	21–34	30–35	37
Nitrate reduction:						
To nitrite	+	+	+	−	+	nd
To N₂	−	−	+	−	−	nd
Growth on:						
Hydrogen sulfide	+	nd	+	+	nd	+
Thiosulfate	+	+	+	−	+	+
Thiocyanate	+	−	+	−	−	+
PbS	nd	nd	nd	+	nd	+
Methylated sulfides	+	−	−	nd	nd	nd
Complex media	−	+[a]	−	−	nd	nd

[a]Unable to grow on common sugars, organic acids or one-carbon organic substrates.

change from transparent to whitish-yellow with sulfur accumulation. Facultatively chemolithotrophic and mixotrophic. Grow autotrophically with sulfur, thiosulfate, or tetrathionate, but not with thiocyanate. Accumulates tetrathionate and trithionate transiently during growth on thiosulfate. Incapable of heterotrophic growth on single-carbon compounds. Grows mixotrophically in thiosulfate media supplemented with tricarboxylic acid cycle intermediates or amino acids. Optimal growth requires both organic and inorganic substances and thiosulfate or sulfur. Facultatively anaerobic; reduces nitrate and produces nitrite in mixotrophic and autotrophic media with thiosulfate or tetrathionate. Ammonium salts, nitrate, urea, glutamate, or aspartate can be used as the nitrogen source.

Optimal temperature 30–35°C, range 15–42°C (no growth at 10 or 45°C). Optimal pH 5.5–6.0, range 5.0–7.0. Isolated from mine water. Distribution unknown.

The mol% G + C of the DNA is: 66–67 (T_m, chemical analysis).

Type strain: IAM 12624.

Additional Remarks: Until 16S rDNA sequence data become available for this species, it cannot be firmly reassigned to the genus *Thiomonas*. Physiologically, the species is more similar to *Thiomonas perometabolis* than to the type species *Thiobacillus thioparus*.

4. **Thiobacillus denitrificans** (ex Beijerinck 1904b) Kelly and Harrison 1989, 1855 emend. Kelly and Wood 2000a, 548[VP]
de.ni.tri' fi.cans. M.L. v. *denitrifico* denitrify; M.L. part. adj. *denitrificans* denitrifying.

Short rods, 0.5 × 1.0–3.0 μm. May be motile by means of a polar flagellum. Under anaerobic conditions, colonies are clear or weakly opalescent when grown on thiosulfate–nitrate agar, On aging, colonies may become white with sulfur. Vigorous nitrogen production when grown under anaerobic conditions, leading to splitting of agar when solid media are used. Facultatively anaerobic. Grows autotrophically and aerobically on thiosulfate or tetrathionate, on which it produces growth yields approximately double those of *T. thioparus* or *T. neapolitanus*. Grows anaerobically on thiosulfate, tetrathionate, or sulfide by using nitrate, nitrite, or nitrous oxide as the terminal electron acceptor. Oxidizes sulfur, sulfide, thiosulfate, tetrathionate, and probably sulfite, but not thiocyanate. Chemostat culture can be switched easily and repeatedly between aerobic and anaerobic growth modes, with adaptation involving derepression of nitrate and nitrite reductase synthesis. Ammonium salts and, in at least some strains, nitrate are used as nitrogen sources.

Obligately chemolithotrophic and autotrophic. Optimal temperature: 28–32°C. Optimal pH: 6.8–7.4. Found in soil, mud, and freshwater and marine sediments, especially under anoxic conditions. Probably very widely distributed.

The original isolation of Beijerinck (1904a) may not have been a pure culture (Vishniac and Santer, 1957), and a viable sample is not available in the Delft Culture Collection (L.A. Robertson, personal communication) or in any other culture collection. Later work has demonstrated unambiguously that Beijerinck's designation was of a legitimate species exhibiting stable physiological character-

istics (Lieske, 1912; Baalsrud and Baalsrud, 1954; Taylor et al., 1971; Justin and Kelly, 1978; Katayama-Fujimura et al., 1982). Earlier claims that the capacity of this organism to denitrify was lost on aerobic subculture and that it was facultatively heterotrophic were erroneous. The name has therefore been revived by Kelly and Harrison (1989, in Validation List No. 31), who suggested a neotype strain. It is now proposed that NCIB 9548 (the strain isolated by White and Hutchinson and used by Justin and Kelly, 1978; AB7, ATCC 23644, JCM 3870) be accepted as this reference strain (Kelly and Wood, 2000a).*

The mol% G + C of the DNA is: 63 (Bd, T_m).
Type strain: ATCC 23644, DSM 12475, NCIB 9548.
GenBank accession number (16S rRNA): AJ243144.

5. **"Thiobacillus plumbophilus"** Drobner, Huber, Rachel and Stetter 1992, 217.
plum.bo' philus. L. neut. n. *plumbum* lead; Gr. v. *philein* to love; M. L. adj. *plumbophilus* loving lead, referring to its ability to grow with lead sulfide as a sole energy source.

Cells are rod-shaped, Gram negative, 0.25 × 3 μm, and motile by one polar flagellum. Optimal growth between 21°C and 34°C, with growth occurring at up to 41°C. Growth between pH 4.0 and 6.5. Strictly chemolithoautotrophic and aerobic. Oxidation of galena (PbS), H_2S, and hydrogen. Sensitive to ampicillin and rifampicin. Possesses 96.5% ubiquinone Q-8. Isolated from a uranium mine in Germany. Insignificant DNA hybridization to *A. ferrooxidans* and *"T. cuprinus"*.

The mol% G + C of the DNA is: 66 (T_m, HPLC).
Deposited strain: DSM 6690.

6. **"Thiobacillus prosperus"** Huber and Stetter 1989, 484.
pros' pe.rus. L. masc. adj. *prosperus* prosperous, referring to its ability to gain precious metals by ore "leaching".

Cells are Gram-negative rods, 0.3 × 3–4 μm. Motile by one polar flagellum. Optimal growth around 37°C and up to 41°C. Growth from 0–3.5% NaCl (some strains tolerate 6% NaCl) and at pH 1.0–4.5. Strictly chemolithoautotrophic and aerobic. Growth on sulfidic ores, like pyrite, sphalerite, chalcopyrite, arsenopyrite, and galena, and on H_2S. Poor growth on elemental sulfur and ferrous iron. Produces sulfuric acid from reduced sulfur compounds. Sensitive to ampicillin and vancomycin. Possesses *meso*-diaminopimelic acid and ubiquinone Q-8. Lives in marine sediments in hydrothermal areas. Insignificant DNA hybridization to *T. ferrooxidans*, *T. thiooxidans*, *T. neapolitanus*, and *T. thioparus*. Member of the class *Gammaproteobacteria*.

The mol% G + C of the DNA is: 64 (T_m, HPLC).
Deposited strain: DSM 5130.
GenBank accession number (16S rRNA): AY034139.

Additional Remarks: "*T. prosperus*" will in due course be removed from the genus, as recent work has shown its closest phylogenetic relatives to be *"Acidihalobacter aerolicus"* (DSM 14174) and *"Acidihalobacter ferrooxidans"* (DSM 14175), with both of which it shares 95% 16S rRNA sequence identity (P.R. Norris, K.B. Hallberg and B. Johnson, personal communication, 2001).

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*Editorial Note: In the original description, the authors erroneously designated the neotype strain as NCIMB 8327.

Order III. **Methylophilales** *ord. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Me.thy.lo.phi.la' les. M.L. masc. n. *Methylophilus* type genus of the order; *-ales* ending to
denote order; M.L. fem. n. *Methylophilales* the *Methylophilus* order.

The order *Methylophilales* was circumscribed for this volume on
the basis of phylogenetic analysis of 16S rRNA sequences; the
order contains the family *Methylophilaceae*.

Description is the same as for the family *Methylophilaceae*.
Type genus: **Methylophilus** Jenkins, Byrom and Jones 1987, 447.

Family I. **Methylophilaceae** *fam. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Me.thy.lo.phi.la' ce.ae. M.L. masc. n. *Methylophilus* type genus of the family; *-aceae* ending to
denote family; M.L. fem. pl. n. *Methylophilaceae* the *Methylophilus* family.

The family *Methylophilaceae* was circumscribed for this volume on
the basis of phylogenetic analysis of 16S rRNA sequences; the
family contains the genera *Methylophilus* (type genus), *Methylo-
bacillus*, and *Methylovorus*.

Aerobic respiratory metabolism. Oxidize methanol but not
methane. Some grow on a limited range of other compounds.
Found in a variety of habitats.

Type genus: **Methylophilus** Jenkins, Byrom and Jones 1987, 447.

Genus I. **Methylophilus** *Jenkins, Byrom and Jones 1987, 447*[VP]

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Me.thy.lo.phi' lus. Fr. n. *méthyle* the methyl radical; Gr. adj. *philos* loving; M.L. masc. n. *Methylophilus* methyl
radical loving.

**When grown on methanol–mineral salts agar or in methanol–
mineral salts liquid medium, the cells are straight or slightly
curved rods, usually 0.3–0.6 × 0.8–1.5 μm, occurring singly or
in pairs**. Gram negative, but the stain is often not taken up well.
Motile by polar flagella or nonmotile. Endospores are absent. **No
cellular inclusions**. No sheaths or prosthecae detected. No cap-
sules formed, but **slime may be produced by some strains**. Col-
onies on methanol–mineral salts agar plates incubated for 2 days
at 30°C or 37°C are **nonpigmented**, circular, 1–2 mm in diameter,
with entire edge, convex, translucent to opaque. Pyocyanin and
fluorescein are not produced. No, or extremely poor, growth on
nutrient agar and in nutrient broth incubated at 30°C or 37°C
for 2 days. No, or extremely poor, growth on blood agar; no
hemolysis. Optimal temperature, 30–37°C; no growth occurs at
4°C or 45°C. Optimal pH, 6.5–7.2. **Aerobic**. **Metabolism respi-
ratory**; very little or no acid is produced from glucose. **Methanol
is oxidized as the sole carbon and energy source by all strains**.
In addition, a limited range of other carbon compounds such
as methylamines, formate, glucose, and fructose may be utilized
as sole carbon and energy sources. Methane is not used. Nutri-
tionally nonexacting; nitrate and ammonium salts serve as nitro-
gen sources. Catalase positive and **oxidase positive**. The fatty acid
composition is primarily of the nonhydroxylated straight-chain
saturated and monounsaturated types with $C_{16:0}$ and $C_{16:1}$ pre-
dominating. The major isoprenoid quinone components are ubi-
quinones with eight isoprene units (Q-8). Isolated from activated
sludge, mud, and river and pond water.

The mol% G + C of the DNA is: 50 (T_m).

Type species: **Methylophilus methylotrophus** Jenkins, Byrom
and Jones 1987, 447.

FURTHER DESCRIPTIVE INFORMATION

Hexulose phosphate synthetase and hexulose phosphate isom-
erase, key enzymes of the ribulose monophosphate pathway
(RMP) for methanol assimilation, are present (Beardsmore et
al., 1982; ElRayes et al., 1991). Methanol dehydrogenase, and
NAD^+-linked formaldehyde and formate dehydrogenases also
occur (Beardsmore et al., 1982). A variant of the ribulose mono-
phosphate cycle of formaldehyde fixation occurs that involves
cleavage of hexose phosphate by 2-keto-3-deoxy-6-phosphoglu-
conate aldolase and a rearrangement sequence involving trans-
ketolase and transaldolase (Beardsmore et al., 1982).

Hydroxypyruvate reductase, isocitrate lyase, malyl-CoA-lyase,
and glyoxylate aminotransferase, which are characteristic of the
serine assimilation pathway, are absent (ElRayes et al., 1991).

A glucose-6-phosphate dehydrogenase is present that is active
with both $NADP^+$ and NAD^+. Two separate 6-phosphogluconate
dehydrogenases occur, one active with both $NADP^+$ and NAD^+
and the other active only with NAD^+ (Beardsmore et al., 1982).

Ammonia is assimilated via the glutamate dehydrogenase
pathway (ElRayes et al., 1991). Acetamide and acrylamide are
hydrolyzed by a cytoplasmic amidase (Silman et al., 1991).

The most positive redox potential ever recorded for a flavin
adenine dinucleotide-containing protein has been measured for
an electron-transfer flavoprotein (ETF) synthesized by *Methylo-
philus methylotrophus* (Byron et al., 1989).

Urea is hydrolyzed to ammonia by a cytoplasmic urease that
is inducible by urea and short-chain amides and repressed by
excess ammonia (Greenwood et al., 1998).

TAXONOMIC COMMENTS

Studies of the type strain by 16S rRNA analysis have placed the
genus in the class *Betaproteobacteria* (Tsuji et al., 1990).

List of species of the genus Methylophilus

1. **Methylophilus methylotrophus** Jenkins, Byrom and Jones 1987, 447[VP]

me.thy.lo.tro′ phus. M.L. n. *methyl* the methyl radical; Gr. adj. *tropho* pertaining to nutrition; M.L. adj. *methylotrophus* methyl radical-consuming.

The cells are motile by single flagella. Colonies on methanol–mineral-salts agar are grayish-white. In addition to growth on methanol as the sole carbon and energy source, good growth occurs on glucose and may or may not occur on methylamines as sole carbon and energy sources. Different strains give different results with fructose as the sole carbon and energy source. Poor growth, which varies between strains, may occur on lactose, sucrose, D-ribose, D-xylose, ethanol, propanol, butanol, acetate, and formate. Acid is not produced from glucose. Acetoin, tested by the Voges–Proskauer method, may or may not be produced. Tween 20, 40, and 60 are hydrolyzed. Tween 80 is not hydrolyzed. Urease is produced. Leucine arylamidase is produced. Phosphatase production is weak and differs between different strains. Sulfatase is not produced. H_2S is not produced. Gelatin is not liquefied. Extracellular DNase and RNase are not produced. 2,3,5-Triphenyltetrazolium chloride (0.01%) is reduced. No growth occurs in the presence of 0.01% potassium tellurite, or with 5% NaCl. Resistant to penicillin, oleandomycin; sensitive to nalidixic acid, streptomycin, and a number of other antibiotics.

The type strain produces significant amounts of a low-viscosity extracellular polysaccharide from methanol under conditions of nitrogen limitation in chemostat culture.

The mol% G + C of the DNA is: about 50 (T_m), 50.3 (T_m) (NCIB 10515[T]), 49.8 (T_m) (ATCC 31226[T]).

Type strain: AS1, ATCC 53528, DSMZ 46235, NCIB 10515.
GenBank accession number (16S rRNA): L15475.

2. **Methylophilus leisingeri** corrig. Doronina and Trotsenko 2001b, 1[VP] (*Methylophilus leisingerii* (sic)) (Effective publication: Doronina and Trotsenko 1994, 529.

lei.sing′ e.ri. M. L. gen. n. *leisingeri* named after the Swiss microbiologist, Thomas Leisinger, who isolated the organism.

Gram-negative rods 0.4–0.6 × 1.1–1.7 μm. Nonmotile (unlike *M. methylotrophus*). A slender polysaccharide capsule is present. Colonies on methanol salt agar are circular, 1–4 mm in diameter, white or pale pink in color, opaque, convex, with even edges and a smooth surface. Temperature range, 10–37°C; optimum, 30–35°C. pH range, 6.5–7.8; optimum, 6.8–7.2. The generation time is 2 h on media with methanol or dichloromethane, 10 h with glucose. No vitamins or other growth factors are required. Cellulose is not digested; gelatin is not liquified; starch is weakly hydrolyzed. Acetoin, indole, hydrogen sulfide, and ammonia are not produced. Oxidase, catalase, and urease positive. Strictly aerobic, although able to reduce nitrates to nitrites. Carbon and energy sources include methanol, dichloro- and dibromomethane, glucose, and galactose; methylated amines are not used. Nitrogen sources include ammonium and nitrate. No growth occurs on media with 3% NaCl.

One-carbon units are assimilated via the 2-keto-3-deoxy-6-phosphogluconate (KDPG) variant of the ribulose monophosphate pathway. The Krebs cycle lacks an α-ketoglutarate dehydrogenase. Glyoxylate shunt enzymes are not present. Ammonium is assimilated via the glutamate cycle; glutamate dehydrogenase is lacking. Cells contain a constitutive methanol dehydrogenase (PQQ) and may produce an inducible dichloromethane dehalogenase and hexokinase. Palmitic ($C_{16:0}$) and palmitoleic ($C_{16:1}$) acids predominate among the cellular fatty acids. Cardiolipin is absent from cellular phospholipids. The major ubiquinone is Q-8. The type strain exhibits a DNA hybridization value of 26% with the type strain of *M. methylotrophus*. The type strain was isolated from wastewater in Switzerland.

The mol% G + C of the DNA is: 50.2 (T_m).
Type strain: VKM B-2013, DM11, DSM 6813.

Genus II. **Methylobacillus** Yordy and Weaver 1977, 254[AL], emend. Urakami and Komagata 1986a, 509

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Meth.yl.o.ba.cil′ lus. Fr. *méthyle* the methyl radical; L. dim. n. *bacillus* a small rod; M.L. masc. n. *Methylobacillus* methyl rodlet.

Rods 0.3–0.6 × 0.8–2.0 μm. **Motile by means of a single polar flagellum or nonmotile.** Gram negative. **Most strains are obligate methylotrophs that grow on one-carbon compounds other than methane;** however, some strains can also use fructose. **Aerobic,** having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. The major cellular fatty acids are straight-chain saturated $C_{16:0}$ and unsaturated $C_{16:1}$. The major quinone is Q-8; Q-7 and Q-9 are minor components.

The mol% G + C of the DNA is: 50–56.

Type species: **Methylobacillus glycogenes** Yordy and Weaver 1977, 254; emend. Urakami and Komagata 1986a, 510.

MAINTENANCE PROCEDURES

Cultures of *M. glycogenes* have been maintained on Medium B[1] at 30°C.

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1. Medium B (Urakami and Komagata, 1986a) contains (per liter of distilled water): $(NH_4)_2SO_4$, 3.0 g; KH_2PO_4, 1.4 g; Na_2HPO_4, 3.0 g; $MgSO_4·7H_2O$, 0.2 g; ferric citrate, 0.03 g; $CaCl_2·2H_2O$, 0.03 g; $MnCl_2·4H_2O$, 0.005 g; $ZnSO_4·7H_2O$, 0.005 g; $CuSO_4·5H_2O$, 0.0005 g; yeast extract, 0.2 g; vitamin solution, 1.0 ml; methanol, 10.0 ml. The vitamin solution contains (per liter of distilled water): biotin, 2 mg; calcium pantothenate, 400 mg; pyridoxine hydrochloride, 400 mg; *p*-aminobenzoic acid, 200 mg; folic acid, 2 mg; inositol, 2 g; nicotinic acid, 400 mg; riboflavin, 200 mg.

DIFFERENTIATION OF THE GENUS *METHYLOBACILLUS* FROM OTHER GENERA

Table BXII.β.73 lists characteristics differentiating *Methylobacillus* from other nonmethane-utilizing methylotrophs.

TAXONOMIC COMMENTS

In this edition of the *Manual*, the genus *Methylobacillus* is placed in the class *Betaproteobacteria*, the order *Methylophilales*, and the family *Methylophilaceae*. This family includes three genera: *Methylophilus*, *Methylobacillus*, and *Methylovorus*.

List of species of the genus Methylobacillus

1. **Methylobacillus glycogenes** Yordy and Weaver 1977, 254[AL]; emend. Urakami and Komagata 1986a, 510.

   gly.co′gen.es. Gr. adj. *glykus* sweet; Gr. v. *gennairo* to produce; Gr. adj. *glycogenes* sweet-producing, intended to mean sugar-producing, glycogen-producing.

   Gram-negative rods with rounded ends, 0.3–0.5 × 0.8–2.0 μm, occurring singly or rarely in pairs. Motile by means of single polar flagella. Do not form spores. Do not form capsules. Do not accumulate poly-β-hydroxybutyrate. No growth occurs in nutrient or peptone broth. Colonies are shiny, smooth, raised, entire, white to light yellow, 1–3 mm in diameter after 3 days at 30°C on methanol-containing agar. Nitrate reduced to nitrite. Methyl red and Voges–Proskauer negative. Do not produce indole, hydrogen sulfide, or ammonia. Do not hydrolyze gelatin or starch. No denitrification. No acid from D-glucose or D-fructose. Utilize methanol but not methane as the sole carbon source. Obligate methylotroph. Do not utilize L-arabinose, D-xylose, D-glucose, D-mannose, galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, soluble starch, succinic acid, citric acid, acetic acid, ethanol, or hydrogen. Variable utilization of D-fructose and methylamine. Utilize ammonia, urea, and nitrate as sole nitrogen sources. Urease and oxidase negative. Catalase positive. Growth at 30°C (most strains also grow at 37°C) and pH 6–8. Most strains do not grow in the presence of 3% NaCl.

   The mol% G + C of the DNA is: 50–56; type strain 54 (Bd).

   Type strain: T-11, ATCC 29475, DSM 5685, JCM 2850, NCIB 11375.

   GenBank accession number (16S rRNA): M95652.

2. **Methylobacillus flagellatus** Govorukhina, Kletsova, Tsygankov, Trotsenko and Netrusov 1998, 631[VP] (Effective publication: Govorukhina, Kletsova, Tsygankov, Trotsenko and Netrusov 1987, 676.)

TABLE BXII.β.73. Characteristics differentiating *Methylobacillus* species from each other and from other nonmethane-utilizing methylotrophs[a,b]

Characteristic	*Methylobacillus glycogenes*	*Methylobacillus flagellatus*	*Acidomonas methanolica*	*Methylobacterium*	*Methylophaga*	*Methylophilus methylotrophus*	*Methylovorus glucosotrophus*	*Xanthobacter*
Methane utilization	–	–	–	D	–	–	–	–
Oxidizes ethanol to acetic acid	–	–	+	–	–	–	–	–
Grows only below pH 5.5	–	–	+	–	–	–	–	–
Na$^+$ or seawater required for growth	–	–	nd	–	+	–	–	–
Poly-β-hydroxybutyrate accumulation in cells	–	nd	nd	+	nd	–	nd	nd
Colony pigmentation	white to light yellow	milky	nd	pink to orange-red	pale pink	none	pink, creamy, or milky	yellow
Known to fix N$_2$	–	–	–	–	–	–	–	+
Urease	nd	–	nd	+	+	+	+	nd
Oxidase	–	+	nd	+ or W	+	+	+	+
Glucose can be used as a carbon and energy source	–	–	nd	D	–	+	+	W
Fructose can be used as a carbon and energy source	d	nd	nd	D	+	v and W	–	+
Starch hydrolysis	–	+	nd	nd	nd	nd	+	nd
Major 1-C pathway:								
Ribulose monophosphate	+	nd	nd	–	+	+	+	nd
Serine	–	nd	nd	+	–	–	–	nd
Ammonia assimilation:								
Glutamate cycle	–	nd	nd	nd	nd	+	+	nd
Glutamate dehydrogenase	+	nd	nd	nd	nd	–	–	nd
Presence of 6-phosphogluconate dehydrogenase (NADP-linked)	+	nd	nd	nd	nd	+	–	nd
Presence of branched C$_{17}$ fatty acids	–	nd	nd	nd	nd	+	–	nd
Presence of diphosphatidyl glycerol	nd	+	nd	nd	–	+	nd	nd

[a]Symbols: see standard definitions; nd, not determined; w, weak.

[b]Data from and Yordy and Weaver (1977), Urakami and Komagata (1986a), Govorukhina et al. (1987), Jenkins et al. (1987), Govorukhina and Trotsenko (1991), Holt et al. (1994), Padden et al. (1997).

fla.gel.la' tus. L. *flagellum;* L. *flagellatum* for attached flagella, flagellated.

Gram-negative rods, 0.5–0.6 × 1.4–1.8 μm. Motile. Reproduce by division. Do not form spores or capsules. Colonies on agar with methanol are round, translucent, with milky color, smooth, convex, with an even edge and viscous consistency. Obligate methylotroph. Methanol and methylamine are used as carbon and energy sources. Methylamine, ammonia salts, dimethylamine, nitrates, and peptone are used as nitrogen sources. Optimal temperature 42°C;

range, 10– 52°C. Optimal pH 7.2–7.3; range 5.8–8.4. Generation time is 2 h. Vitamins are not required. No acid or gas is produced from glucose. Urease, oxidase, and catalase positive. Nitrate reduced to nitrite. Hydrolyze starch but not cellulose, gelatin, or Tween 80. Major fatty acids are $C_{16:0}$ and $C_{16:1}$. The ratio of *cis*-vaccenic acid to palmitoleic acid is 0.32.

The mol% G + C of the DNA is: 55.5.
Type strain: KT, ATCC 51484, DSM 6875, VKM B-1610.
GenBank accession number (16S rRNA): M95651.

Genus III. **Methylovorus** *Govorukhina and Trotsenko 1991, 161*[VP]

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Me.thyl.o' vo.rus. Fr. *méthyle* the methyl radical; L. v. *voro* to consume; M.L. adj. *methylovorus* methyl-consuming.

When grown on methanol–mineral salts medium, cells are straight or slightly curved rods, usually 0.5–0.6 × 1.0–1.3 μm, occurring singly or in pairs. Gram negative. **Motile** by a single polar flagellum. Endospores are absent. No complex intracellular membranes. No sheaths or prosthecae detected. No capsules formed, but slime may be produced by some strains. Colonies on methanol–mineral salts agar incubated for 2 days at 30°C are circular, 1–2 mm in diameter, with entire edge, convex, and translucent to opaque; may be pink, creamy, or milky in color. Pyocyanin and fluorescein not produced. Cells multiply by binary fission. No aggregation or pigmentation occurs in liquid medium. No growth or extremely poor growth occurs on nutrient agar and in nutrient broth at 30–37°C; no growth occurs under an atmosphere of CH_4/O_2 or $H_2/CO_2/O_2$. Optimal pH for growth, 7.0–7.2; temperature, 35–37°C. **Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. **Only methanol and glucose are used as carbon and energy sources.** In addition, some strains are able to grow slowly on methylated amines and betaine. Nitrates, ammonium salts, methylated amines, glutamate, and peptone serve as nitrogen sources. Acetoin, indole, H_2S, and NH_3 are not produced in test medium. Milk is not hydrolyzed. **Oxidase positive.** Urease and catalase positive. Peroxidase variable. Arginine dihydrolase negative. Strains may hydrolyze starch, but not cellulose, gelatin, or Tween 80. Acid, but not gas, is produced from glucose. Isolated from activated sludge, mud, soil, and pond water.

The mol% G + C of the DNA is: 56–57.

Type species: **Methylovorus glucosotrophus** Govorukhina and Trotsenko 1991, 162.

FURTHER DESCRIPTIVE INFORMATION

All strains assimilate methanol carbon through the ribulose monophosphate (RuMP) pathway and ammonia via the glutamate cycle (glutamate synthase and glutamine synthetase). Neither α-ketoglutarate dehydrogenase nor glyoxylate shunt enzymes are present. The fatty acid composition is primarily of the

nonhydroxylated straight-chain saturated and monounsaturated types with $C_{16:0}$ and $C_{16:1\,\omega7}$. Branched C_{17} fatty acids are absent. The major phospholipids are phosphatidylethanolamine and phosphatidylglycerol. The strains also possess diphosphatidylglycerol.

DIFFERENTIATION OF THE GENUS *METHYLOVORUS* FROM OTHER GENERA

Table BXII.β.74 indicates some features that differentiate *Methylovorus* from *Methylophilus* and *Methylobacillus*.

TAXONOMIC COMMENTS

Results of DNA–DNA hybridization and 5S rRNA sequencing studies on some strains of obligately and restricted facultatively methylotrophic bacteria support the establishment of *Methylovorus* as a genus separate from other methylotrophs (Wolfrum and Stolp, 1987; Bulygina et al., 1993). The genus is closely related to *Methylobacillus* and *Methylophilus* and belongs to the class *Betaproteobacteria*.

TABLE BXII.β.74. Characteristics differentiating *Methylovorus* from related genera[a,b]

Characteristic	*Methylovorus*	*Methylophilus*	*Methylobacillus*
Presence of:			
6-Phosphogluconate dehydrogenase (NADP-linked)	−	+	+
Branched C_{17} fatty acids	−	+	−
Diphosphatidyl glycerol	+	−	+
Assimilation of NH_3 occurs by:			
Glutamate cycle (glutamate synthase and glutamine synthetase)	+	+	−
Glutamate dehydrogenase	−	−	+
Mol% G + C of the DNA	56–57	50–53	50–56

[a]For symbols see standard definitions.

[b]Data from Govorukhina and Trotsenko (1991).

List of species of the genus Methylovorus

1. **Methylovorus glucosotrophus** Govorukhina and Trotsenko 1991, 162[VP]

    glu.co.so.tro' phus. M.L. n. *glucosum* glucose; Gr. adj. *tropho* pertaining to nutrition; M.L. adj. *glucosotrophus* glucose-consuming.

    The characteristics are as described for the genus. In addition, generation times when grown on methanol and glucose are 2 and 17 h, respectively. Vitamins and co-factors are not required.

    The mol% G + C of the DNA is: 56–57 (T_m).
    Type strain: 6B1, DSM 6874, UCM B-1475.

Order IV. **Neisseriales** *ord. nov.*

TONE TØNJUM

Neis.se.ri.a' les. M.L. fem. n. *Neisseriaceae* type family of the order; *-ales* ending to denote an order; M.L. fem. pl. n. *Neisseriales* the *Neisseriaceae* family.

Organisms are coccal, coccoid, or distinctly rod-shaped, occurring singly, in pairs, in masses, or in short chains. Cells of *Aquaspirillum* are helical, while *Simonsiella* and *Alysiella* may exhibit a characteristic multicellular micromorphology. Endospores are not formed. Gram-negative, but there may be a tendency to resist decoloration. Flagella and swimming motility are absent except in the genus *Aquaspirillum*. The cells are nonmotile in liquid media but surface-bound motility ("twitching motility") is frequently observed. Fimbriae (pili) are often present.

All species grow aerobically. Strains of all recognized species usually have an optimal growth temperature of approximately 32–36°C. Capsules may be present. Colonies of most genera are not pigmented, with the exception of strains of *Vogesella* and *Chromobacterium*. Several species have complex growth factor requirements, while some species grow readily in simple defined media containing a single organic carbon and energy source.

The mol% G + C of the DNA is: 46–67.

Type genus: **Neisseria** Trevisan 1885, 105.

TAXONOMIC COMMENTS

The order *Neisseriales* comprises a major branch of the *Betaproteobacteria* with the single family *Neisseriaceae* as the type family. *Neisseriaceae* consists of the type genus *Neisseria* as well as the heterogeneous genera (in alphabetical order) *Alysiella, Aquaspirillum, Catenococcus, Chromobacterium, Eikenella, Formivibrio, Iodobacter, Kingella, Microvirgula, Prolinoborus, Simonsiella, Vitreoscilla,* and *Vogesella.* In order of decreasing relatedness to the type genus *Neisseria* the genera included in *Neisseriaceae* include *Kingella, Eikenella, Alysiella, Simonsiella* (Rossau et al., 1986, 1989; Dewhirst et al., 1989), *Microvirgula* (Patureau et al., 1998), *"Laribacter"* (Yuen et al., 2001), *Vogesella* (Grimes et al., 1997), *Vitreoscilla* (Joshi et al., 1998), *Chromobacterium* (Dewhirst et al., 1989), *Aquaspirillum* (Rossau et al., 1989; Wen et al., 1999), *Prolinoborus* (Pot et al., 1992b), *Formivibrio* (Hippe et al., 1999), and *Iodobacter* (Fig. BXII.β.61). Other entities that, based on 16S rRNA phylogenetic analysis, show the closest relatedness to *Neisseriales* are *Taylorella* spp. encompassing *Taylorella equigenitalis* and *Taylorella asinigenitalis* (Bleumink-Pluym et al., 1993; Jang et al., 2001) and *Pelistega europaea* (Vandamme et al., 1998), through their relationship to *Iodobacter fluviatilis;* these genera appear closer to the *Neisseriaceae* type genus *Neisseria* than does the genus *Catenococcus.* However, the genera *Taylorella* and *Pelistega* are currently placed in the new family *Alcaligenaceae* in the order *Burkholderiales.*
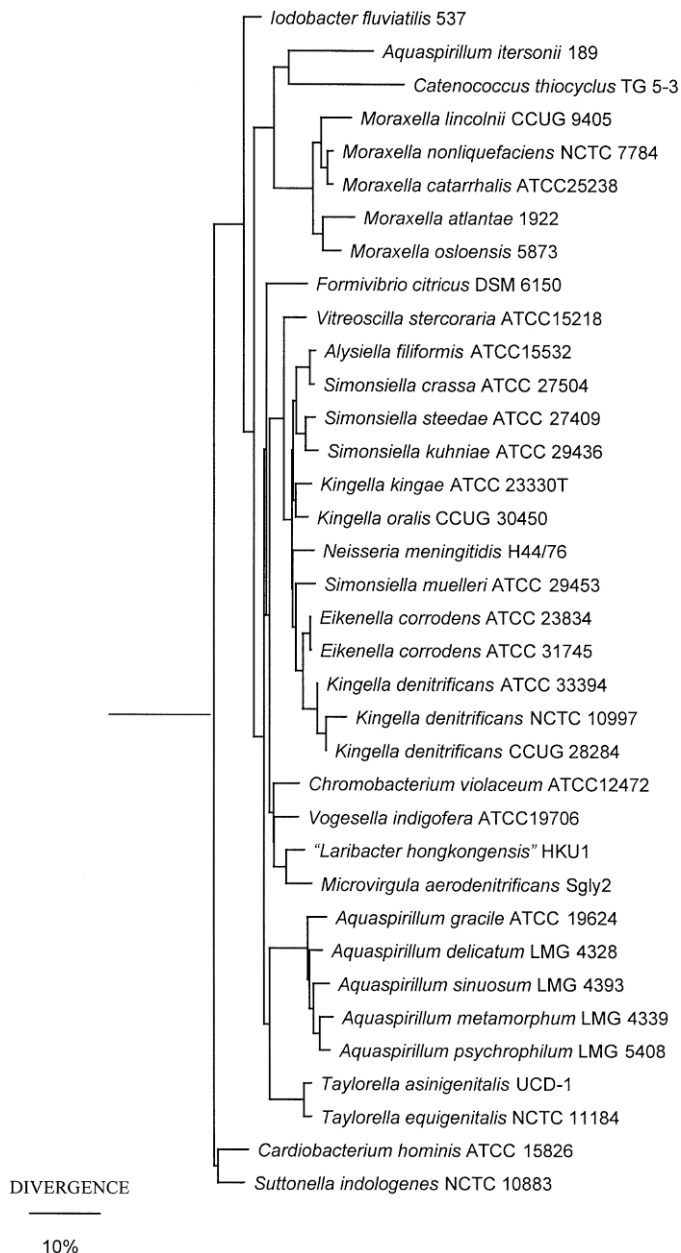

FIGURE BXII.β.61. Phylogenetic neighbor-joining tree of the family *Neisseriaceae* based on 1355 nucleotide positions of the 16S rRNA genes. *Oligella urethralis* CIP 103 116 was used as an outgroup (K.K. Garborg, S.A. Frye, B. Pettersson and T. Tønjum, unpublished studies).

Family I. **Neisseriaceae** Prèvot 1933, 119[AL] emend. Dewhirst, Paster and Bright 1989, 265

TONE TØNJUM

Neis.se.ri.a' ce.ae. M.L. fem. n. *Neisseria* type genus of the family; -*aceae* ending to denote a family; M.L. fem. pl. n. *Neisseriaceae* the *Neisseria* family.

Organisms are coccal, occurring singly, in pairs, or in masses, often with adjacent sides flattened (different planes of division), or distinctly rod-shaped or coccoid (one plane of division), frequently occurring in pairs or short chains. Cells of *Aquaspirillum* are helical, while *Simonsiella* and *Alysiella* may exhibit a characteristic multicellular micromorphology. Endospores are not formed. Gram negative, but there may be a tendency to resist decolorization. Flagella and swimming motility are absent. The cells are nonmotile in liquid media but surface-bound motility ("twitching motility") is frequently observed. Fimbriae (pili) are often present.

All species grow aerobically. Strains of all recognized species usually have an optimal growth temperature of approximately 32–36°C. Capsules may be present. Colonies are not pigmented. Several species have complex growth factor requirements, while some species grow readily in simple defined media containing a single organic carbon and energy source.

All strains except those of *Formivibrio* are oxidase positive. Usually catalase positive. Indole is not produced. True waxes are not present. Fatty acid profiles show the presence of mainly unbranched saturated and mono- or di-unsaturated fatty acids composed of 16 and 18 carbon atoms. Most strains reside indigenously on mucosal membranes of humans and animals, although environmental species were recently included in this family.

The mol% G + C of the DNA is: 46–67.

Type genus: **Neisseria** Trevisan 1885, 105.

FURTHER DESCRIPTIVE INFORMATION

Since the 1980s, mainly as a result of the application of new molecular techniques, major changes have occurred within the taxonomy and classification of the family *Neisseriaceae.* This family comprises a major branch of the *Betaproteobacteria.* Studies with DNA–DNA and DNA–rRNA hybridizations, and subsequently 16S rRNA sequence comparisons, have redefined the relationships among species belonging to the *Neisseriaceae* (Dewhirst et al., 1989; Rossau et al., 1989; Enright et al., 1994; Tønjum et al., 1995a; Harmsen et al., 2001). Findings from DNA-mediated transformation to streptomycin resistance, DNA base composition data, DNA–DNA and DNA–rRNA hybridization, and rRNA sequencing have revealed differences that mandate the separation of the genera *Moraxella* and *Acinetobacter* (belonging to the *Gammaproteobacteria*) from *Neisseriaceae* (Stackebrandt et al., 1988b; Dewhirst et al., 1989). In addition, using transformation analysis, the four coccal species of the "false neisseriae" (previously *"N. catarrhalis"*, *N. caviae, N. ovis,* and *N. cuniculi*) have been transferred from *Neisseria* to the *Gammaproteobacteria* (Catlin, 1970; Bøvre, 1979). The moraxellae, including the "false neisseriae", psychrobacters, and acinetobacters, were grouped to form the new family *Moraxellaceae* (Catlin, 1991; Rossau et al., 1991; Pettersson et al., 1998b), leaving the genera *Neisseria, Kingella,* and *Simonsiella,* the genospecies *Eikenella corrodens* and *Alysiella filiformis,* and the CDC groups EF-4 and M-5 as members of *Neisseriaceae* (Fig. BXII.β.61; Dewhirst et al., 1989; Rossau et al., 1989). Cur-

rently, *Aquaspirillum, Catenococcus, Chromobacterium, Formivibrio, Iodobacter, Microvirgula, Prolinoborus, Vitreoscilla,* and *Vogesella* are also assigned to *Neisseriaceae.* Table BXII.β.75 lists some differential characteristics of the genera of the family.

Many species of *Neisseriaceae* contain strains that are highly competent for genetic transformation (see Table BXII.β.77 in the *Neisseria* chapter). In addition to representing the most important form of genetic exchange causing genomic heterogeneity (Spratt et al., 1992), transformation also provides a tool for definite identification of numerous, although not all, species of the *Neisseriaceae,* and also forms an important basis for allocation of species within the genera of this family (Bøvre, 1980; Bøvre and Hagen, 1981; Tønjum et al., 1995b).

Genetic taxonomy in *Neisseriaceae* was pioneered by Catlin and Cunningham in 1961 when they demonstrated close relationships between most *Neisseria* species (except the species then misnamed *"N. catarrhalis"*) and by Bøvre and Henriksen in 1962. That a genus may contain both coccal and rod-shaped species was shown for genus *Neisseria* by the detection of the rod-shaped organism known as *Neisseria elongata* (Bøvre and Holten, 1970). More recently, another rod-shaped organism, *N. weaveri,* has been included in the genus *Neisseria* (Andersen et al., 1993; Holmes et al., 1993). The genus *Kingella* was proposed by Henriksen and Bøvre in 1976 for the organism known previously as *Moraxella kingae* (now *Kingella kingae*), and was subsequently enlarged by the proposed inclusion of two more species, *K. denitrificans* and *K. indologenes* (Snell and Lapage, 1976). The genus *Kingella* now consists of the species *K. kingae, K. denitrificans,* and *K. oralis* (Dewhirst et al., 1993), while *K. indologenes* was transferred to the family *Cardiobacteriaceae* as *Suttonella indologenes* (Dewhirst et al., 1990). A slight genetic affinity between *K. kingae* and *N. elongata* has been demonstrated by genetic transformation and DNA–DNA hybridization (Bøvre and Holten, 1962; Tønjum et al., 1989).

The cellular lipid composition of *Neisseriaceae* is characterized by the absence of branched fatty acids. The quantitative and qualitative distributions of straight-chain fatty acids, including hydroxy acids, generally distinguish the genera as genetically circumscribed and can also be important in differentiation among several species (Jantzen et al., 1974, 1975; Bøvre et al., 1976). True waxes, i.e., simple esters of fatty alcohols and fatty acids, are absent in the genera *Neisseria* and *Kingella,* while activities corresponding to the enzymes thymidine phosphorylase, nucleoside deoxyribosyltransferase, and thymidine kinase have been shown to be present in the genus *Kingella,* but absent in the genus *Neisseria* (Jyssum and Bøvre, 1974). Carbonic anhydrase has been detected in all *Neisseria* species (Berger and Issi, 1971).

TAXONOMIC COMMENTS

The taxonomic placement of *Neisseriaceae* corresponds to rRNA superfamily III according to DNA–rRNA hybridizations performed by Rossau et al. (1986, 1989). Based on 16S rDNA phylogeny, *Neisseriaceae* consists of the type genus *Neisseria,* as well as the genera (in alphabetical order) *Alysiella, Aquaspirillum, Caten-*

TABLE BXII.β.75. Differential characteristics of the genera of the family *Neisseriaceae*[a]

Characteristic	*Neisseria*	*Alysiella*	*Aquaspirillum*	*Chromobacterium*	*Eikenella*	*Formivibrio*	*Iodobacter*	*Kingella*	*"Laribacter"*	*Microvirgula*	*Simonsiella*	*Vitreoscilla*	*Vogesella*
Cell morphology:													
Cocci	+	−	−	−	−	−	−	−	−	−	−	−	−
Rods	+[b]	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+[c]	+	+	+	+	+
Catalase test	+	+		+	−	−		−		+	+	+	
Acid from glucose	[+]	+	D	+	−		−	+	−	−	[+]		−
Nitrite reduction	+	−		+	+			+		+	[+]		+
Mol% G + C of DNA	46.5–58.0	44–48	56–62	65–68	56–58	59	50–52	47–55		68	65	40–52	65–69

[a]Symbols: +, positive for the majority of strains and some strains of each species; [+], positive for all strains of the majority of species (only one species uniformly negative); D, positive and negative species (or strains of *Acinetobacter*) about equally represented; −, all strains negative.

[b]Only two species, *N. elongata* and *N. weaveri*, consist of rods.

[c]*K. denitrificans* may be negative or weakly positive with the least sensitive test reagent, dimethyl-*p*-phenylenediamine, whereas it is distinctly positive when tetramethyl-*p*-phenylenediamine is used. For other organisms of this table (including *K. kingae*) the result is the same with either reagent.

ococcus, Chromobacterium, Eikenella, Formivibrio, Iodobacter, Kingella, Microvirgula, Prolinoborus, Simonsiella, Vitreoscilla, and *Vogesella*. In order of decreasing relatedness to the type genus *Neisseria* the genera included in *Neisseriaceae* encompass *Kingella, Eikenella, Alysiella, Simonsiella* (Rossau et al., 1986, 1989; Dewhirst et al., 1989), *Microvirgula* (Patureau et al., 1998), *"Laribacter"* (Yuen et al., 2001), *Vogesella* (Grimes et al., 1997), *Vitreoscilla* (Joshi et al., 1998), *Chromobacterium* (Dewhirst et al., 1989; Martin and Brimacombe, 1992; Duran and Menck, 2001), *Aquaspirillum* (Rossau et al., 1989; Wen et al., 1999), *Prolinoborus* (Pot et al., 1992b), *Formivibrio* (Hippe et al., 1999), and *Iodobacter* (Fig. BXII.β.61). Other entities that, based on 16S rRNA phylogenetic analysis, show the closest relatedness to *Neisseriales* are *Taylorella* spp., encompassing *Taylorella equigenitalis* and *Taylorella asinigenitalis* (Bleumink-Pluym et al., 1993; Jang et al., 2001), and *Pelistega europaea* (Vandamme et al., 1998) through their relationship to *Iodobacter fluviatilis*. These genera appear closer to the genus *Neisseria* than does the genus *Catenococcus*. The phylogenetic maps obtained by 16S rDNA sequence analysis show that species within *Neisseriaceae* are polyphyletic. This is due to the high frequency of horizontal gene transfer by transformation combined with frequently occurring recombination events and a relatively high spontaneous mutation rate (Spratt et al., 1992). These factors contribute to the limitations of the use of 16S rDNA sequences as the basis for taxonomy in this bacterial family.

Microvirgula aerodenitrificans (Patureau et al., 1998) are denitrifying and very motile curved rods isolated from activated sludge. Although phenotypically similar to *Comamonas testosteroni*, phylogenetic analysis based on the 16S rRNA sequence shows that *Microvirgula aerodenitrificans* is most closely related to the genus *Vogesella*. Another recent addition to the genera in *Neisseriaceae* is the genus *"Laribacter"*, represented by *"Laribacter hongkongensis"* (Yuen et al., 2001). This bacterium was isolated from the blood and empyema of a cirrhotic patient. The cells are seagull shaped or spiral rods. Phylogeny based on 16S rDNA sequence analysis shows that *"L. hongkongensis"* is most closely related to *Microvirgula aerodenitrificans, Vogesella indigofera*, and *Chromobacterium* species, respectively.

Vogesella indigofera (Grimes et al., 1997) has blue-pigmented colonies with a metallic copper-colored sheen. These blue-pigmented bacteria were isolated from freshwater samples, and numerical analysis of morphological and biochemical characteristics has revealed 90.0% relatedness between *Vogesella* spp. and *Burkholderia cepacia* and *Janthinobacterium lividum*. A phylogenetic analysis in which both 5S rRNA and 16S rRNA were used also revealed that the *Vogesella* strains were closely related to *Chromobacterium violaceum*, but sufficiently distinct to warrant placement in a separate genus, *Vogesella*, named in honor of Otto Voges, who first isolated and described this blue-pigmented eubacterium in 1893.

Selected strains/biovars currently classified as *Aquaspirillum* spp., *Aquaspirillum gracile, Aquaspirillum delicatum, Aquaspirillum sinuosum, Aquaspirillum metamorphum,* and *Aquaspirillum psychrophilum* also belong to the *Neisseriaceae* (Wen et al., 1999), together with *Prolinoborus* (Pot et al., 1992b). *Iodobacter fluviatilis* (previously *Chromobacterium fluviatile*) (Wen et al., 1999) and *Catenococcus thiocycli* (partial 16S rDNA sequence submitted by Tourova, T.P. and Kuznetsov, B.B., Moscow, Russia) are the entities most distantly related to the type genus *Neisseria* (Fig. BXII.β.61).

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Genus I. **Neisseria** *Trevisan 1885, 105*[AL]

Tone Tønjum

Neis.se' ri.a. M.L. fem. n. *Neisseria* named after Albert Neisser, who discovered the etiological agent of gonorrhea in the pus of patients in 1889.

Cocci 0.6–1.9 μm in diameter, occurring singly but often in pairs with adjacent sides flattened; two species (*Neisseria elongata* and *N. weaveri*) are exceptions and consist of short rods 0.5 μm wide, often arranged as diplobacilli or in chains. Division of the coccal species is in two planes at right angles to each other, sometimes resulting in tetrads. Capsules and fimbriae (pili) may be present. Endospores are not present. Gram negative, but there is a tendency to resist decolorization. Swimming motility does not occur and flagella are absent. Aerobic. Some species produce a yellow carotenoid pigment. Some species are nutritionally fastidious and hemolytic. Optimal temperature, 35–37°C. Oxidase positive. Catalase positive except most strains of *N. elongata*. Carbonic anhydrase is produced by all species. All species reduce nitrite except *N. gonorrhoeae* and *N. canis*. *Neisseria* spp. produce acid from carbohydrates by oxidation, not fermentation. Chemoorganotrophic. Exotoxins are not produced. Some species are saccharolytic. Inhabitants of the mucous membranes of mammals. Some species are primary pathogens of humans. The genus *Neisseria* belongs to the family *Neisseriaceae* of the *Betaproteobacteria*.

The mol% G + C of the DNA is: 48–56.

Type species: **Neisseria gonorrhoeae** (Zopf 1885) Trevisan 1885, 106 (*Merismopedia gonorrhoeae* Zopf 1885, 54.)

FURTHER DESCRIPTIVE INFORMATION

Cells of most *Neisseria* spp. are cocci that divide in two planes at right angles to each other, often resulting in the formation of diplococci and tetrads. However, cells of the species *N. elongata* and *N. weaveri* are short, slender rods (Bøvre and Hagen, 1981; Andersen et al., 1993). *Neisseria* spp. produce oxidase and catalase, carbonic anhydrase, and nitrite reductase, but do not produce thymidine phosphorylase, nucleoside deoxyribosyl transferase, or thymidine kinase. In contrast to *Moraxella* spp., the cell walls of *Neisseria* spp. do not contain true waxes. Summaries of the occurrence and isolation (Bøvre and Hagen, 1981), phylogeny and taxonomy (Bøvre and Hagen, 1981), biochemistry, physiology, and identification (Morse and Knapp, 1989; Knapp and Koumanis, 1999), and clinical significance (Cartwright, 1995; Knapp and Koumanis, 1999) of neisseriae are available.

Phylogeny and classification The genus *Neisseria* contains 17 species and biovars that may be isolated from humans including six species that may be isolated from animals (Table BXII.β.76). The taxonomy of the species of human origin has undergone many changes; these and the names by which individual species have been known have been summarized (Rossau et al., 1989; Tønjum et al., 1995b; Knapp and Koumanis, 1999). *Neisseria* spp. may be identified by phenotypic characteristics including their patterns of acid production from carbohydrates, production of polysaccharide from sucrose, and reduction of nitrate (Table BXII.β.76), as well as 16S rDNA sequence analysis (Fig. BXII.β.62).

Based on conventional characterization and 16S rDNA sequence analysis, *Neisseria* spp. can be divided into three major groups. The first group includes the closely related species *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. cinerea*, and *N. polysaccharea*. Strains of these species grow as nonpigmented, translucent colonies. Members of the second group, which consists of *N. subflava* (including biovar flava, biovar perflava, and biovar

subflava), *N. sicca*, and *N. mucosa*, referred to as the saccharolytic species, usually grow as opaque, yellow-pigmented colonies on solid media. Some strains of *N. subflava* biovar perflava have translucent colonies. Although asaccharolytic, *N. flavescens* grows as yellow, opaque colonies and appears to be a member of this group (Kingsbury, 1967; Rossau et al., 1989). The third group contains *N. elongata* and the animal species *N. weaveri*, *N. denitrificans*, *N. animalis*, *N. canis*, *N. macacae*, *N. iguanae*, and *N. dentiae*. However, based on 16S rDNA sequence analysis, *N. denitrificans* is just as related to *Simonsiella muelleri* and *Kingella denitrificans* as to *Neisseria* spp., and *N. canis* and the other animal neisserial species are related to *K. kingae* and *K. oralis*. The genetic basis for interrelatedness of strains of *Neisseria* and other genera of the *Neisseriaceae* is addressed below.

Cell morphology Although the genus *Neisseria* has traditionally consisted only of cocci (Fig. BXII.β.63), two rod-shaped species, *N. elongata* and *N. weaveri*, are currently included in the genus.

Cell wall composition Neisserial cell surface variation serves as an adaptive mechanism that can modulate tissue tropism, immune evasion, and survival in the changing host environment.

Outer membrane The outer membrane of the neisseriae is typical for a Gram-negative bacterium and consists of phospholipids, several proteins, and LPS. The gonococcal outer membrane is more permeable to hydrophobic compounds such as fatty acids, detergents, and certain antibiotics than the membranes of other Gram-negative bacteria (Miller et al., 1977). This may be due in part to the presence of phospholipids in the outer leaflet of the outer membrane (Lysko and Morse, 1981).

Outer membrane proteins *N. gonorrhoeae* and *N. meningitidis* possess several outer membrane proteins that have been studied at the molecular level and found to be analogous to one another. The nomenclature of these proteins has been devised to reflect the genetic relatedness of these organisms (Hitchcock, 1989).

PORINS The gonococcal (protein I, PI) and meningococcal (class 1, 2, and 3 proteins) outer membranes contain trimeric proteins that form hydrophilic pores (porins) termed Por (Newhall et al., 1980a; Douglas et al., 1981; Derrick et al., 1999; Müller et al., 1999). The porin function is necessary for the survival of the bacterium, allowing nutrients and other solutes to enter the cell. The neisserial porin proteins are important as serotyping antigens, putative vaccine components, and for their proposed role in the intracellular colonization of humans. Gonococci express one of two classes of porins, termed PIA and PIB or PorA and PorB (Sandstrøm et al., 1982), which are encoded by alleles of the same gene (Gotschlich et al., 1987b; Carbonetti et al., 1988). Antigenic heterogeneity in the PIA and PIB molecules provides the basis for gonococcal serotyping. In contrast to gonococci, many meningococcal strains express two porin molecules, one encoded by *porA* (designated the PorA or class I protein) and a second encoded by *porB* (designated as the PorB or class II/III). PorB is the meningococcal counterpart of gonococcal porin with class II corresponding to PIA and class III corresponding to PIB, while PorA/class I expression is unique to meningococci. Evidence of interspecies recombination of *porB*

TABLE BXII.β.76. Differential characteristics of the species of the genus *Neisseria*[a]

Characteristic	N. gonorrhoeae	N. animalis	N. canis	N. cinerea	N. denitrificans	N. dentiae	N. elongata	N. flavescens	N. iguanae	N. lactamica	N. macacae	N. meningitidis	N. mucosa	N. polysaccharea	N. sicca	N. subflava	N. weaveri
Shape of cells:																	
Cocci	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−
Rods[b]	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Arrangement of cells:																	
Pairs	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	−
Tetrads	−	+	−	−	−	−	−	+	+	−		−	−	+	+	+	−
Short chains	−	−	−	−	−	−	+	−	−	−		−	−	−	−	−	+
Yellowish pigment	−	−	+	D	D	(+) 71	weak	+	+	+	+	−	D	+	D	+	−
Hemolysis on blood agar:																	
Sheep	−	−	−	−	−	−	−	−	+w	D	+	−	−		D	−	+
Horse	−	−	d	−	−	−	−	−	+	−	+	−	−	−	D	−	−
Rabbit	−	−	−	−	−	−	−	−		−	+	−	−		D	D	−
Human	−	−	−	−	−	−	−	−		−	−	−	−	−	D	D	−
Acid produced from:																	
Glucose	+	−	−	−	+	+	D	−	+w	+	+	+	+	+	+	+	−
Maltose	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−
Fructose	−	−	−	−	+	+	−	−		−	+	−	+	−	+	D	−
Sucrose	−	−	−	−	+	+	−	−	−w	−	+	−	+	−	+	D	−
Mannose	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Lactose	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−
Nitrate reduction	−[c]	−	+	−	+	−	−	−	+	−	+	−	+	+	−	−	−
Nitrite reduction	−	−	−[c]	+	+	+	+	+	+	+	+	−[c]	+	−	+	+	+
Gas from nitrite	−	−	−	+	+	+	+	+		+	+	−	+	−	+	+	−
Synthesis of polysaccharide (iodine test)	−	−	−	−	−	−	−	+		−	+	−	+	+	+	D[d]	−
Tributyrin hydrolysis	−	−	−	−	−	+	−	−		−	−	−	−	−	−	−	−
Mol% G + C of DNA	50–53	ND	50	49–51	56	ND	53	46–50	51–52	52	50–51	50–52	50–52	53–56	49–52	48–51	51–52

[a]Symbols: +, positive for the majority of strains of each species; (+), positive for all strains of the majority of species, only one strain known to be negative; −, negative for the majority of strains of each species; D, positive and negative strains about equally represented; −w, reported as both negative and weakly positive for most strains of each species; +w, reported as both positive and weakly positive for most strains of each species; ND, not determined. Data compiled from: Hollis et al. (1969), Reyn (1974), Riou (1977), Catlin (1978), Morello and Bonhoff (1980), Bøvre and Hagen (1981), Hoke and Vedros (1982a), and Andersen et al. (1993).

[b]Only two species, *N. elongata* and *N. weaveri*, consist of rods.

[c]Nitrite in low concentrations can be reduced by *N. gonorrhoeae* and by serogroups A, D, and Y of *N. meningitidis* (Berger, 1970).

[d]*N. subflava* biovar perflava is positive; the reaction differs among strains of *N. subflava* biovar subflava.
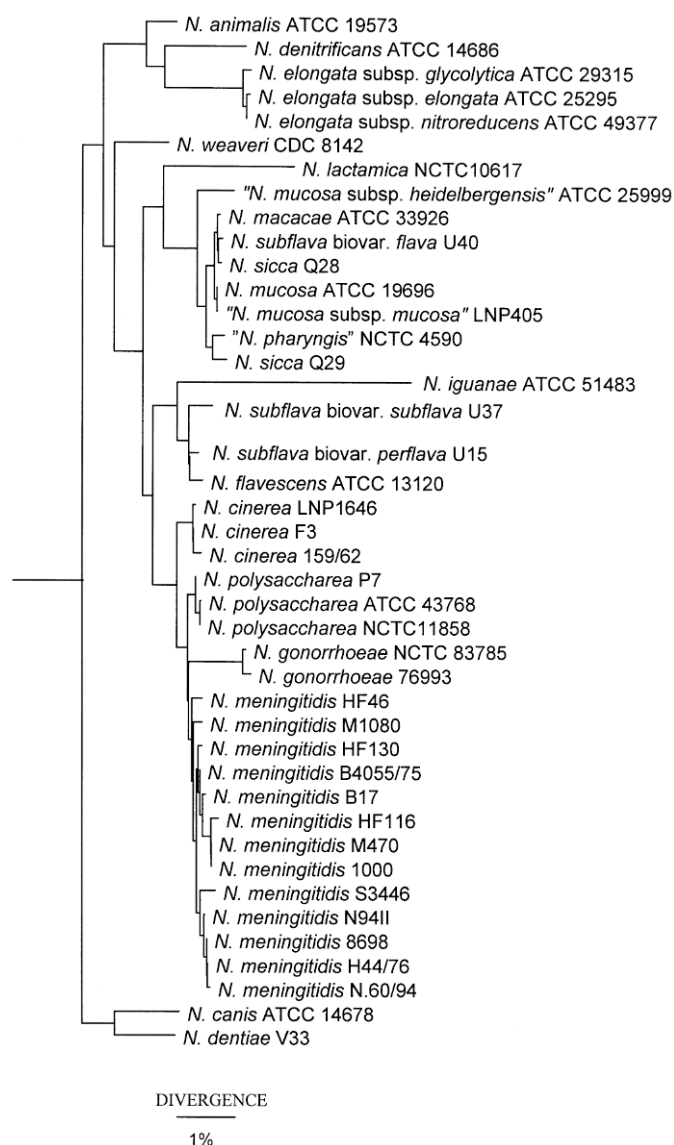
FIGURE BXII.β.62. Neighbor-joining tree of the genus *Neisseria* based on 1355 nucleotide positions of the 16S rRNA genes. *Moraxella nonliquefaciens* NCTC 7784 was used as an outgroup (Reproduced with permission from K.K. Garborg., S.A. Frye, B. Pettersson and T. Tønjum, unpublished studies).

antibodies (Lehmann et al., 1999). PorA/class I protein appears to be an important component for noncapsular meningococcal vaccines, but like PorB/class II/III is polymorphic due to horizontal gene exchanges (Bart et al., 1999). Moreover, the efficacy of vaccines composed solely of PorA/class I protein may be compromised because strains failing to express the PorA/class I molecule have been shown to arise due to 1) phase variation owing to slipped strand mispairing within its promoter region (van der Ende et al., 1995), 2) complete deletions of the gene (van der Ende et al., 1999), and 3) gene inactivation via insertion of IS1301 (Newcombe et al., 1998). Phase variation appears to account for the absence of PorA expression in most strains (Bart et al., 1999).

Several studies have demonstrated the association of particular PI serotypes with either disseminated or localized gonococcal infections (Cannon et al., 1983; Sandstrøm et al., 1984; Brunham et al., 1985). Strains of *N. gonorrhoeae* isolated from patients with disseminated disease (PIA strains), and strains of *N. meningitidis* were more efficient at porin insertion than strains isolated from a mucosal site (PIB strains). PIA has also been shown to be specifically associated with increased bacterial invasiveness for human cells, which may explain its association with invasive disease (van Putten et al., 1998). Treatment with PI also decreased the ability of human neutrophils to exocytose granules and affected other cellular functions (Haines et al., 1988). PI has been considered as a potential vaccine candidate against gonococcal disease. Antibodies against PI are effective in both bactericidal and opsonic assays (Virji et al., 1986, 1987), and infection with one PI serotype appears to provide protection against subsequent infection with the same serotype (Buchanan et al., 1980). However, even the use of a meningocccal hexavalent PorA vaccine candidate in a clinical trial did not result in adequate protection (Rouppe van der Voort et al., 2000).

OPACITY-ASSOCIATED (OPA) PROTEINS Both *N. gonorrhoeae* and *N. meningitidis* express a family of closely related but size-variable surface-exposed outer membrane proteins termed opacity-associated or Opa proteins. The gonococcal proteins have been named protein IIs while those from meningococci are referred to as class 5 proteins. A similar protein has been identified in *N. lactamica* and *N. cinerea* (Aho et al., 1987). Most, but not all, gonococcal opacity-associated proteins are associated with pronounced colony opacity because they increase the aggregation of gonococcal cells and thereby contribute to autoagglutination and adherence (Blake et al., 1995). There is no relationship between colony opacity and expression of the opacity-associated proteins in *N. meningitidis* (Hagman and Danielsson, 1989). With regard to function, gonococcal and meningococcal opacity-associated protein expression have been demonstrated to promote adherence and uptake into mammalian cells by virtue of their ability to engage cell surface receptors including CD66 (Chen et al., 1997a) and proteoglycan receptors (van Putten and Paul, 1995).

The opacity-associated proteins undergo phase variation, are immunogenic, and appear to have an important role in bacterial adherence and invasion. The *in vivo* importance of opacity-associated protein expression for neisserial infection is suggested by the findings that gonococci recovered after urogenital infection are typically Opa$^+$, as are bacteria recovered after the inoculation of human volunteers with transparent (Opa$^-$) bacteria (Swanson et al., 1988). In *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica*, 11, 3–4, and 2 unlinked chromosomal alleles, respec-

has been documented in the isolation of a meningococcal stain whose *porB* allele was virtually identical to that of gonococcal allelle (Vazquez et al., 1995). Although gonococci fail to express PorA/class I protein, they carry a complete but defective copy of the *porA* gene (Feavers and Maiden, 1998). The PorB of *N. gonorrhoeae* is able to translocate from the outer bacterial membrane into host cell membranes where it modulates the infection process (Müller et al., 1999). Most evidence suggests that meningococcal PorB/class II/III proteins behave analogously in function and structure to the gonococcal counterpart, while PorA/class I is structurally distinct but with overlapping function. For example, meningococcal strains lacking PorA/class I protein can be constructed as long as they express PorB/class II/III protein and vice versa (Tommassen et al., 1990). In the case of both porins, immunization has been demonstrated to be capable of engendering bactericidal (van der Voort et al., 1996) and opsonic

FIGURE BXII.β.63. Electron microscopic image of negatively stained *Neisseria meningitidis* strain M1080. Bar = 0.25 mm.

tively, have been identified that encode distinct opacity-associated protein variants (Meyer and van Putten, 1989). Each of these is a complete gene with a functional promoter and is transcribed (Stern and Meyer, 1987). Phase variable expression results from slipped strand mispairing DNA rearrangements, which occur at high frequencies and alter the number of pentanucleotide CTCTT coding repeat units in the N-terminal part of the open reading frames (Murphy et al., 1989). The opacity-associated proteins of *N. gonorrhoeae* and *N. meningitidis* can undergo phase variation during natural infection (Zak et al., 1984; Tinsley and Heckels, 1986).

Opa proteins also display antigenic variability and diversity within strains occurring by recombinational reassortment, which has been documented both in gonococci (Connell et al., 1988) and meningococci (Hobbs et al., 1998).

Opc The meningococcal Opc outer membrane protein and its gene were first identified in a genetic cloning study using antibodies to the meningococcal Class 5C protein (Olyhoek et al., 1991). Further analysis revealed, however, that Opc is structurally and antigenically unrelated to the Class 5 proteins (Merker et al., 1997). Most meningococcal strains are capable of expressing Opc, and intrastrain phase variation occurs owing to changes in the number of contiguous cytosine residues that separate the *opc*-10 promoter and the start codon sequences (Sarkari et al., 1994). Although originally thought to be meningococcal-specific, gonococci carry an allele of *opc* in their genome and Opc is weakly expressed in gonococci (Zhu et al., 1999). Meningococcal Opc promotes binding to and uptake by mammalian cells by virtue of binding proteoglycan receptors (de Vries et al.,

1998). The potential benefits of a humoral immune response to Opc remain unclear (Thiesen et al., 1997).

H.8 protein The DNA sequence predicts that the H.8 protein is a lipoprotein based on the presence of a lipoprotein signal-peptide processing site and an N-terminal cysteine residue. The predicted protein is 71 amino acids in length and is composed entirely of 13 repeats of the AAEAP consensus sequence that was also present in another neisserial protein, the lipid-modified azurin (Gotschlich et al., 1987a; Kawula et al., 1987). This protein is not essential for the growth and survival of gonococci in a complex medium, and the function of this protein remains unknown.

PilQ PilQ was first identified as the outer membrane protein-macromolecular complex (OMP-MC), a major protein component of the outer membrane suggested to account for about 10% of its protein mass (Newhall et al., 1980b; Hansen and Wilde, 1984). This protein is required for type IV pilus biogenesis in the pathogenic neisseriae and was renamed PilQ based on its homology to the *Pseudomonas aeruginosa* PilQ protein (Drake and Kommey, 1995; Tønjum et al., 1998). Neisserial mutants expressing defective forms of the protein are devoid of pili and pilus-associated phenotypes. The PilQ complex has a molecular mass of 800–900 kDa and is composed of 12 identical subunits of 76–80 kDa (Tsai et al., 1989; Drake and Koomey, 1995; Tønjum et al., 1998). The C-terminus of PilQ shares identity with members of a large family of proteins associated with membrane translocation of macromolecular complexes, termed secretins. PilQ appears to function in pilus biogenesis in its complex form by serving as a pore through which pili are extruded. The PilQ

monomer exhibits in its N-terminus a polymorphic region in which an octapeptide repeat occurs 4–7 times in *N. meningitidis* and only 2–3 times in *N. gonorrhoeae*. Antibodies against PilQ are produced during a natural infection (Tønjum and Koomey, personal communication), and PilQ antibodies are bactericidal for both homologous and heterologous gonococcal and meningococcal strains in a complement-dependent assay system (Corbett et al., 1986; Tønjum et al., 1998).

PILC A 110-kDa protein termed PilC was originally identified by virtue of its co-purification with the neisserial pilus fibers has also been identified in the neisserial outer membrane (Jönsson et al., 1991). Neisserial mutants failing to express PilC were reported to show a dramatic reduction in piliation (Jönsson et al., 1991; Nassif et al., 1994). More recently, PilC has been implicated as the molecule responsible for pilus-associated epithelial cell adherence (Rudel et al., 1995a, b).

OUTER MEMBRANE PROTEINS RELATED TO IRON METABOLISM Growth of gonococci and meningococci under iron-limiting conditions leads to the increased production of a variety of outer membrane or periplasmic proteins, collectively termed Irps (iron repressed proteins) (Norqvist et al., 1978). Consistent with the notion that levels of available iron are low in mammalian tissue and fluids, studies of seroconversion following disease indicate that Irps are expressed *in vivo* (Ferreiros et al., 1994). Irps are involved in iron uptake and their expression is coordinately regulated by the action of the iron-dependent regulatory protein Fur (Thomas and Sparling, 1996). Gonococci and meningococci express surface proteins engaged in specific uptake of iron from human transferrin, lactoferrin, and hemoglobin, and, in most cases, proteins found in these two species in each uptake system are structurally and functionally related (Schryvers and Stojiljkovic, 1999). Uptake from transferrin and lactoferrin involves expression of two large proteins, a lipoprotein (Tbp2 and LbpB) and a TonB dependent integral membrane protein (Tbp1 and LbpA), which act in concert to bind their respective ligand (Pettersson et al., 1994). In both systems, expression of the TonB dependent integral membrane proteins is required for function, while that of the lipoprotein is not (Anderson et al., 1994; Pettersson et al., 1998a).

Only 50% of gonococcal strains produce a functional lactoferrin receptor (Biswas and Sparling, 1995), and, as such, the ability to utilize lactoferrin as an iron source is not necessary for gonococcal infection. In contrast, transferrin receptor activity appears to be essential since mutants lacking Tbp1/2 are incapable of colonization in the human urethral challenge model (Cornelissen et al., 1998). Given their surface exposure and expression *in vivo*, meningococcal Irps have been examined for their abilities to engender bactericidal or opsonic antibodies (Lissolo et al., 1995; Ala'Aldeen and Borriello, 1996; Lehmann et al., 1999). Like other surface-exposed molecules, transferrin binding proteins display heterogeneity, which arises due to genetic exchange (Rokbi et al., 1997; Legrain et al., 1998), but such diversity may not automatically preclude their utility as protective immunogens since broadly cross-reactive antibodies against Tbps have also been reported to restrict growth under conditions of iron limitation by virtue of blocking the interaction with transferrin (Ala'Aldeen, 1996).

OTHER MEMBRANE PROTEINS Rmp is a highly conserved, reduction modifiable outer membrane protein that is found only in pathogenic neisseriae (Wolff and Stern, 1995). It is so named because its migration in SDS-PAGE is altered following treatment with reducing agents and was previously designated as Protein III (PIII) in gonococci and the class 4 protein in meningococci. Although mutants lacking Rmp have no discernible phenotypes, the protein does co-purify with the Por proteins (Wetzler et al., 1992b). In addition, antibodies directed against Rmp appear to block the bactericidal activity of antibodies directed toward other outer membrane proteins (Munkley et al., 1991), which may account for the observation that Rmp antibodies are associated with an increased susceptibility to gonococcal infection (Plummer et al., 1993).

Several other neisserial outer membrane proteins have been described. Some of these proteins appear to be both highly conserved and surface exposed. NspA is a conserved membrane protein that is reported to elicit protective antibody responses against *N. meningitidis* serogroups A, B, and C in mice (Martin et al., 1997). Although originally found in meningococci, it has also recently been shown to be expressed by *N. gonorrhoeae* strains (Plante et al., 1999).

Lipo-oligosaccharide Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria. The LPS of enteric bacteria such as *E. coli* consists of a lipid A moiety, a core oligosaccharide, and a repeating O-antigen polysaccharide. In contrast, the corresponding molecule of the pathogenic *Neisseria* spp. lacks the repeating O-antigen polysaccharides and is therefore termed lipo-oligosaccharide (LOS) (Mintz et al., 1984; Yamasaki et al., 1994). Differences in LOS composition appear to account for the difference in M_r. The oligosaccharide component consists of 1) a partially conserved and highly substituted basal oligosaccharide that branches at a heptose residue; 2) a linear segment consisting of $(hexose)_n$ residues that determines the length of the oligosaccharide; and 3) terminal sequences that are similar to those of glycosphingolipids (Griffiss et al., 1988; Mandrell et al., 1988). The resemblance of the lactoneoseries glycolipids in gonococcal (Yamasaki et al., 1994) and meningococcal (Tsai et al., 1998) LOS to human glycolipids appears to represent a form of host mimicry that may play a role in immune evasion (Moran et al., 1996). Gonococci expressing these LOS forms appear to be selected for *in vivo* and may have reduced infective doses (Schneider et al., 1991, 1995). Differences in the chemical composition of individual oligosaccharides relate to their ability to inhibit the killing of serum-sensitive strains of gonococci (Griffiss et al., 1987). *N. meningitidis* releases large amounts of the potent LOS through blebs or lysis. The oligosaccharide of neisserial LOS can be bound by host cell-expressed lectins and, therefore, might also contribute to bacterial adhesion (Apicella et al., 1986; Porat et al., 1995) and invasion (Song et al., 2000d).

Gonococci have the ability to produce a variable set of the oligosaccharide components of LOS at a high frequency. This variation occurs primarily due to phase variable expression of tandemly arrayed glycosyl transferase genes that ensue from slipped strand mispairing within poly-G tracts (Yang and Gotschlich, 1996). The lactoneoseries glycolipids in gonococcal LOS are also able to become modified *in vivo* by sialylating the oligosaccharide with host-derived CMP-*N*-acetyl neuraminic acid (CMP-NANA) (Nairn et al., 1988; Parsons et al., 1989). The enzyme responsible for this modification, sialyl transferase, is of gonococcal origin. The sialylation of the LPS results in the conversion of a serum-sensitive organism to one that is serum-resistant (Parsons et al., 1989) and is one of several mechanisms responsible for this phenomenon in gonococci. The sialylated

LPS appears to be rapidly lost after growth *in vitro* in the absence of CMP-NANA and may explain the type of resistance referred to as unstable serum resistance (Ward et al., 1970; Wetzler et al., 1992a). Curiously, sialylation *in vitro* prior to urethral challenge in men has been reported to reduce infectivity (Schneider et al., 1996). Meningococcal serogroup B and C strains are able to endogenously sialylate LOS since they have sialyl transferase and produce CMP-NANA as a substrate for capsule biosynthesis (Tsai et al., 1998). In contrast to other Gram-negative species, meningococcal mutants lacking LOS are viable (Steeghs et al., 1998). This remarkable finding may be attributable to compensatory changes in the phospholipid composition.

Immunoglobulin M (IgM) class antibodies directed against LPS epitopes are responsible for the complement-dependent bactericidal activity of normal human serum for serum-sensitive strains of *N. gonorrhoeae* (Apicella et al., 1986). The LPS from serum-resistant strains also contains bactericidal epitopes; however, normal human serum often lacks antibodies to these particular epitopes (Schneider et al., 1985). Nevertheless, antibodies to these epitopes are present in convalescent serum samples from patients with disseminated infections (Rice and Kasper, 1977).

Fimbriae (pili) Fimbriae (pili) are hairlike filamentous appendages emanating several micrometers from the bacterial cell surface. Fimbriae have been found on the surface of *N. gonorrhoeae* (Jephcott et al., 1971; Swanson et al., 1971), *N. meningitidis* (DeVoe and Gilchrist, 1975; McGee et al., 1977), and the nonpathogenic *Neisseria* spp., including *N. perflava* and *N. elongata* (Wistreich and Bakter, 1971; Frøholm et al., 1973; Bøvre et al., 1977). A PCR-based screen detected homology to the conserved N-terminal region of the pilus expression locus PilE in 12 out of 15 *Neisseria* species investigated, including all human commensal isolates (Aho et al., 2000).

Neisserial pili are ordered arrays of polymerized protein subunits termed pilin, with a molecular weight that varies between strains in the range of 16–22 kDa (Robertson et al., 1977; Brinton et al., 1978; Buchanan, 1978). Pili from *N. gonorrhoeae* and *N. meningitidis* are antigenically and structurally similar (Hermodson et al., 1978; Olafson et al., 1985; Potts and Saunders, 1988). However, meningococcal pili can be divided into two classes: those cross-reacting with the SM1 monoclonal antibody that recognizes a conserved epitope in gonococcal pili (class I), and those not showing this cross-reactivity (class II). The pili demonstrated on a number of commensal *Neisseria* spp. are homologous to the class II pili of *N. meningitidis* (Aho et al., 2000). The neisserial prepilins at short leader sequences and proximal 30 amino acids (Meyer et al., 1984; Potts and Saunders, 1988) show a high degree of homology with pilins of other Gram-negative human pathogens including *P. aeruginosa* (Strom and Lory, 1986), *Vibrio cholerae* (Faast et al., 1989), enterotoxigenic *E. coli* (ETEC) (Giron et al., 1994), and enteropathogenic *E. coli* (EPEC) (Giron et al., 1991), as well as opportunistic pathogens in the genera *Eikenella* (Tønjum et al., 1993) and *Moraxella* (Frøholm and Sletten, 1977; Tønjum et al., 1991). Collectively, this family of surface appendages has been termed type IV pili (Ottow, 1975) or Tfp (Wolfgang et al., 1998b).

These related pilus colonization factors appear to be essential for infectivity and disease. Pili mediate the attachment of *N. gonorrhoeae* to a wide range of different cell types, including tissue culture cells (Swanson, 1973; Heckels, 1989), vaginal epithelial cells (Mardh et al., 1975), fallopian tube epithelium (Ward et al., 1974), and buccal epithelial cells (Punsalang and Sawyer,

1973). Pili also mediate the attachment of *N. meningitidis* to nasopharyngeal cells (Stephens and McGee, 1981). Evidence for the critical roles of gonococcal and meningococcal pili can be found in the invariable recoveries of piliated organisms from primary cultures (Jyssum and Lie, 1965; Kellogg et al., 1986; Swanson et al., 1987) and the capacities of these structures to undergo antigenic variation (Tinsley and Heckels, 1986; Swanson et al., 1987). Pili appear to be multifunctional organelles. In addition to adherence, their expression is associated with other phenotypes that may be relevant to the host-parasite interaction. Pilus expression is correlated with high level (sequence-specific) competence for DNA transformation with frequencies of transformation being reduced 1000-fold in nonpiliated mutants (Sparling, 1966; Frøholm et al., 1973; Seifert et al., 1990; Zhang et al., 1992; Drake and Koomey, 1995; Freitag et al., 1995; Tønjum et al., 1995b; Drake et al., 1997). The consensus DNA uptake sequence recognized (5′-GCCGTCTGAA) is distributed throughout the gonococcal and meningococcal genomes (Goodman and Scocca, 1988).

Pilus expression is also associated with multicellular behavior. Bacterial autoagglutination (independent of that associated with Opa proteins) is a pilus-dependent phenomenon (Swanson et al., 1971). Although flagella and motility in liquid media are absent in *Neisseria* species, a form of bacterial surface translocation termed "twitching motility" is only displayed by piliated organisms (Henrichsen, 1975b; Swanson, 1978; Wolfgang et al., 1998a). In *P. aeruginosa*, twitching motility is proposed to be a consequence of pilus retraction (Bradley, 1980) and mutants that retain piliation but have lost twitching motility have been shown to carry mutations in a gene designated *pilT* (Whitchurch et al., 1991). Likewise, neisserial PilT mutants do not display twitching motility (Wolfgang et al., 1998b), and laser tweezer analysis of gonococci supports the notion that twitching motility is a consequence of pilus retraction (Merz et al., 2000).

Neisserial pilus expression is subject to both phase and antigenic variation. Gonococcal pilus variation results from homologous recombination between a single complete pilin gene or expression locus (*pilE*) and multiple partial pilin gene copies or silent alleles (*pilS*) (Haas and Meyer, 1986; Swanson et al., 1986; Koomey et al., 1987), and an analogous mechanism is thought to operate in meningococcal pilus variation (Aho and Cannon, 1988; Perry et al., 1988; Blake et al., 1989). Antigenic variation results from the unidirectional transfer of genetic information from the variant-encoding *pilS* genes to the active expression locus. The pilin amino acid changes reflect the nucleotide changes in *pilE*, which result from nonreciprocal RecA-dependent recombination events with numerous silent loci, *pilS*. The most convincing results show that productive pilin gene rearrangements in *N. gonorrhoeae* mainly arise by gene conversion and not by transformation (Zhang et al., 1992). The frequency of phase variation (on–off) from p^+ to p^- (10^{-3}) is much higher than reversion from p^- to p^+ (10^{-6}) (Swanson et al., 1987; Swanson and Koomey, 1989). The expression of pili is therefore often lost on repeated nonselective subculture. The high-frequency changes in the primary amino acid sequence of the pilin subunit alter pilus expression and aid in adjusting attachment abilities and the avoidance of the host immune response. The frequently occurring antigenic variation may result in phase variation, and loss of fimbriation may occur. Cultivating *N. gonorrhoeae*, *N. meningitidis*, and *N. elongata* in special growth conditions such as pellicle formation and selective subculture based on the observation of colony morphology will maintain pilus expression

(McGee et al., 1979; Swanson et al., 1987; Swanson and Koomey, 1989).

The fimbriae are produced and exported to the surface by the action of a multi-component machinery homologous to type II secretion systems (Tønjum and Koomey, 1997). A number of genes encoding products involved in neisserial pilus biogenesis have been identified (Tønjum and Koomey, 1997). By chromosomal mapping (Dempsey et al., 1995), it has become clear that these genes are distributed throughout neisserial genomes. The machinery dedicated to pilus biogenesis comprises, in addition to the pilin subunit itself, cytoplasmic and cytoplasmic membrane components as well as periplasmic and outer membrane components. A locus carrying the genes *pilF*, *pilD*, and *pilG* related to pilus biogenesis encodes cytoplasmic and cytoplasmic membrane components. Mutants failing to express functional PilD, PilF, or PilG were nonpiliated and showed over a 1000-fold decrease in frequencies of competence for natural transformation (Freitag et al., 1995; Tønjum et al., 1995b). Based on homology to *P. aeruginosa* PilD, the neisserial PilD is thought to function as a prepilin peptidase and methylase of the first amino acid in the mature protein (Strom et al., 1991; Freitag et al., 1995; Tønjum et al., 1995b). PilD is the only component engaged in pilus biogenesis that to date has a defined function (Nunn and Lory, 1991). The role of PilF is still obscure, but its identity with other related molecules (Pugsley, 1993; Sandkvist et al., 1995) and evidence from studies of its homologues in *P. aeruginosa* suggests that it may function as an ATPase or kinase (Turner et al., 1993). The deduced amino acid sequence of PilG reveals the absence of a signal sequence and three membrane spanning domains, which indicates it is a cytoplasmic membrane protein. The exact functional role of PilG remains to be established, but it has been proposed that the homologous PilC in *P. aeruginosa* may be needed for optimal localization or stabilization of PilD (Koga et al., 1993; Tønjum et al., 1995b). Gonococcal PilT, a protein homologous to PilF and belonging to a large family of molecules sharing a highly conserved nucleotide binding domain motif, has been shown to be dispensable for pilus biogenesis but essential for twitching motility and competence for genetic transformation (Wolfgang et al., 1998b).

Outer membrane components engaged in pilus biogenesis characterized to date are PilQ, PilP, and PilC. The PilQ protein, which is essential for type IV pilus assembly, is a member of the secretin family (see above) (Russel, 1998). The common functions served by the secretin homologues appear to involve the translocation of large, macromolecular complexes across the outer membrane. PilQ of the pathogenic neisseriae forms a multimer composed of 10–12 subunits that is resistant to SDS and heating (Newhall et al., 1980a), and the conserved C-terminal residues are necessary for expression of the multimerized form and function in pilus extrusion (Drake and Koomey, 1995; Tønjum et al., 1998). The meningococcal PilQ protein is constitutively expressed in abundant amounts. Analysis of the quaternary structure of meningococcal PilQ by transmission electron microscopy and self-rotation analysis suggest that PilQ is organized as a ring of 12 identical subunits (Collins et al., 2001, 2003). The *pilP* gene, immediately upstream of *pilQ*, encodes a lipoprotein that is predicted to be localized to the outer membrane, and PilP⁻ mutants are nonpiliated (Drake et al., 1997). It seems that PilP is required for stable expression of the multimerized form of PilQ. Gonococcal PilP⁻ as well as PilQ⁻ mutants release PilC (Drake et al., 1997). PilQ and PilC may interact during the terminal stages of pilus biogenesis (Drake et al., 1997; Wolfgang et al., 2000).

The presence or absence of fimbriae is of no taxonomic value in the genus, but is indirectly important because of the use of the associated property competence for natural transformation in qualitative and semiquantitative assays (see below) (Bøvre and Hagen, 1981; Tønjum et al., 1995b).

Secreted proteins *N. meningitidis* and *N. gonorrhoeae* produce IgA1-protease, which degrades and inactivates immunoglobulin of the IgA1 subtype found in mucosal secretions and serum (Koomey and Falkow, 1987).

Peptidoglycan The peptidoglycan composition of those species of *Neisseria* that have been analyzed is typical of other Gram-negative bacteria. The peptidoglycans of *N. gonorrhoeae* and *N. perflava* belong to chemotype 1 and are composed of muramic acid, glucosamine, alanine, diaminopimelic acid, and glutamic acid in an approximate molar ratio of 1:1:2:1:1, respectively (Martin et al., 1973; Hebeler and Young, 1976). The percentage of peptide cross-linking in gonococcal peptidoglycan was found to be approximately 41%, which is relatively high for a Gram-negative bacterium (Rosenthal et al., 1980). *N. gonorrhoeae* and *N. flava* peptidoglycans are extensively O-acetylated (Martin et al., 1973; Blundell and Perkins, 1981). O-acetylated peptidoglycan is more resistant to lysozyme and other human peptidoglycan hydrolases (Rosenthal et al., 1980; Blundell and Perkins, 1981), and this resistance to hydrolases might enable gonococci to persist *in vivo* or potentiate the biological effects in pathogenesis of peptidoglycan *in vivo*.

Polysaccharides Several *Neisseria* sp. are known to produce polysaccharide capsules and exopolysaccharides. The acidic and hydrophilic capsular polysaccharides provide the surface charge and humid environment that are critical for survival of the bacteria in aerosol droplets. Serogrouping of *N. meningitidis* is based on antigenic differences in capsular polysaccharides, and presently recognized serogroups include A, B, C, H, I, K, L, X, Y, Z, W135, and 29E (Frasch, 1989). The capsular polysaccharide may be a homopolymer or a heteropolymer composed of a monosaccharide-glycerol, disaccharide, or trisaccharide repeating unit. 1,2-Diacylglycerols have been identified as components of meningococcal capsular polysaccharides (Gotschlich et al., 1981). It has been postulated that the presence of these hydrophobic moieties may be responsible for the association of the capsular polysaccharide with the outer membrane of the meningococcus, giving rise to the structure recognized as a capsule. The disease-associated serogroup A capsule is composed of mannoseamine-phosphate, while the disease-associated serogroups B, C, Y, and W-135 all have sialic acid in their capsular polysaccharide. Sialic acid-containing polysaccharides confer resistance to host complement-mediated attack mechanisms (Jarvis and Vedros, 1987). *N. meningitidis* serogroup B strains are a predominant cause of meningococcal disease in the developed world. The serogroup B polysaccharide is a homopolymer of alpha-2,8 polyneuraminic acid and is poorly immunogenic, probably because of immunotolerance resulting from cross-reactivity between this polysaccharide and polysialic acid expressed on host neural cell adhesion molecules (NCAMs) (Finne et al., 1983). *N. meningitidis* serogroup B capsular polysaccharide is also chemically and immunologically identical to capsular polysaccharide in *E. coli* K-1 and surface polysaccharide found in a large proportion of *Moraxella nonliquefaciens* strains (Bøvre et al., 1983; Krambovitis et al., 1987).

The genes encoding all the enzymes necessary for capsular poly-saccharide biosynthesis in *N. meningitidis* serogroup B are located on a 5-kb DNA fragment within the chromosomal capsule (*cps*) gene cluster (Frosch et al., 1989). It contains genes encoding enzymes required for capsular polysaccharide biosynthesis in the cytoplasm, for phospholipid substitution, and for the production of proteins whose function is the translocation of polysaccharide across the inner and outer bacterial cell membranes. The full-size capsular polysaccharide with a phospholipid anchor is syn-thesized intracellularly, and the lipid modification is a strong requirement for translocation of the polysaccharide to the cell surface (Frosch and Müller, 1993). Regulation of capsule ex-pression is shown to be performed through slipped strand mis-pairing of a polyC stretch in the *siaD* gene (Hammerschmidt et al., 1996).

It seems evident that the capsule of *N. meningitidis* functions as a virulence factor, since the virulence of this organism de-pends, in part, on the antiphagocytic properties of its capsule (Masson and Holbein, 1983). In general, meningococcal capsular polysaccharides are immunogenic in humans; antibodies di-rected against the capsule are bactericidal. However, serogroup B capsular polysaccharide is poorly immunogenic in humans, possibly because of its similarity to sialic acid moieties in human tissues. Furthermore, the serogroup A and C capsules are poorly immunogenic in infants and young children (Lieberman, 1996). *N. gonorrhoeae* does not have a capsule that can be confirmed by electron microscopy, using India ink (Melly et al., 1979) or wheat germ agglutinin (Frasch, 1980). However, *N. gonorrhoeae* pro-duces a high-molecular-weight extracellular polyphosphate (Noe-gel and Gotschlich, 1983), and a similar polyphosphate is also produced by *N. meningitidis*, *N. lactamica*, *N. sicca*, and *N. flava* (Noegel and Gotschlich, 1983). It has been postulated that the extracellular polyphosphate may serve the function of a capsule in gonococci.

N. perflava possesses a novel system for the synthesis of a gly-cogen-like polysaccharide. The mechanism involves the transfer of the glucosyl moiety of sucrose to a 1,4-α-D-glucan by the en-zyme amylosucrase (Okada and Hehre, 1974). A similar D-glucan is produced by *N. polysaccharea* (Riou et al., 1986), *N. canis*, *N. cinerea*, *N. cuniculi*, *N. denitrificans*, *N. sicca*, and *N. subflava* (MacKenzie et al., 1977, 1978a, b, c) when grown on a medium containing sucrose. A galactosamine polymer has been isolated from the cell walls of *N. sicca* (Adams and Chaudhari, 1972; Wagner et al., 1973). The function of this polymer is presently unknown.

Some bacterial species produce a starch-like polysaccharide from sucrose that stains dark blue-purple to black with iodine. Among the *Neisseria* spp., *N. polysaccharea*, *N. subflava* biovar per-flava, *N. mucosa*, *N. sicca*, and *N. flavescens* produce polysaccharide from sucrose. This test is invaluable for differentiating between strains of *N. meningitidis* (polysaccharide-negative) and *N. poly-saccharea* (polysaccharide-positive); as many as 25% of organisms identified as nontypable *N. meningitidis* strains were found to be *N. polysaccharea* when tested for polysaccharide production.

Colony morphology and cultural characteristics Colony mor-phology varies with the species and ranges from small smooth, transparent, butyrous colonies to wrinkled, dry, adherent colo-nies (Catlin, 1978; Swanson and Koomey, 1989). Colony mor-phology has been particularly useful in characterization of *N. gonorrhoeae* and *N. elongata*, since agglutinating and aggregating bacterial properties display various forms of colony consistency

and edge, as well as spreading and/or corroding growth (Kellogg et al., 1963; Reyn et al., 1971; Swanson and Koomey, 1989). The autoagglutination of bacterial cells into multicellular aggregates induces clumping in broth and variations in colony morphology that can be observed in oblique light in a dissecting stereoloupe/microscope (Juni and Heym, 1977). Important components af-fecting autoagglutination are pilus expression, opacity proteins, and LPS. Colonies of cells expressing pili and opacity proteins are wrinkled and well defined with a clear edge, while colonies from non-piliated cells have a less defined edge and are smoother and more glistening (Swanson and Koomey, 1989; Koomey et al., 1991). Opaque and transparent variants of the colony types have been described (Swanson, 1978). The autoagglutinating colonies are typically isolated from the urethra of infected males and the cervix of females (see review by James and Swanson, 1978; Swan-son et al., 1987). The autoagglutination properties can be cam-ouflaged in strains expressing a capsule.

Colonies of *N. meningitidis* are larger than those of gonococci (≥1.0 mm in diameter) and are smooth and moist with a glis-tening surface and entire edge (Morello and Bonhoff, 1980). The colony morphology of meningococci is difficult to distin-guish, probably due to the capsule giving rise to mucoid colonies. Colonies of *N. lactamica* closely resemble those of meningococci, but may be less moist and smaller (Morello and Bonhoff, 1980). Some strains of *N. lactamica* produce a yellow pigment (Hollis et al., 1969). A yellowish green pigment is also produced by *N. subflava*, *N. flavescens*, and *N. sicca*. *N. elongata* may also have a slight yellow tinge due to pigment production. Pigmentation of neisseriae varies with growth conditions, and quantitative analysis of the extracted pigment indicates that it is of little taxonomic value (Berger, 1961a; Hoke and Vedros, 1982c).

Nutrition and growth conditions *Neisseria* species other than *N. meningitidis* and *N. gonorrhoeae* will grow on plain nutrient agar at 35–37°C. *N. meningitidis* requires mineral salts, lactate, a few amino acids, and glutamic acid as a carbon source (Reyn, 1974; Catlin, 1978). Cystine is required by approximately 10% of the strains (Catlin, 1978), and some strains can be adapted to grow with ammonium salts as the sole nitrogen source (Jyssum, 1959). *N. gonorrhoeae* is more fastidious and requires glutamine for pri-mary isolation of approximately 20% of strains and co-carbox-ylase for 1% of strains (Reyn, 1974). Several defined media have been developed for growth of *N. gonorrhoeae* (Catlin, 1973; LaScolea and Young, 1974; Wong et al., 1980b). Iron is an es-sential growth factor for *N. gonorrhoeae* (Kellogg et al., 1963) and its availability in culture media influences the virulence of both meningococci and gonococci (Payne and Finkelstein, 1975). The optimal temperature for growth of *Neisseria* spp. is 35–37°C with a range of 22–40°C, except for *N. meningitidis* and *N. gonorrhoeae*, which grow poorly or not at all below 30°C. A high relative hu-midity (50%) is beneficial to the growth of all species, and CO_2 (3–10%) is required for the growth of gonococci and enhances the growth of meningococci on solid media. After many passages, laboratory strains become less fastidious in their growth require-ments than fresh isolates. See Catlin (1977) for a detailed review of gonococcal nutritional requirements.

N. meningitidis and *N. gonorrhoeae* do not survive long after the cessation of growth, and decreases in viability appear to be related to cellular lysis (autolysis) (Morse and Bartenstein, 1974). Several peptidoglycan-degrading enzymes have been described in *N. gonorrhoeae*, including D-alanine carboxypeptidase (or endopep-tidase) (Davis and Salton, 1975; Chapman and Perkins, 1983),

N-acetylmuramyl-L-alanine amidase (Hebeler and Young, 1976), transglycosylase (Rosenthal, 1979; Sinha and Rosenthal, 1980), exo-*N*-acetyl-glucosaminidase (Chapman and Perkins, 1983), and endo-*N*-acetylglucosaminidase (Gubish et al., 1982; Chapman and Perkins, 1983). It is likely that some of these enzymes normally have a biosynthetic role in cell growth, but that under nongrowth conditions (i.e., no cell wall biosynthesis) enzyme activity results in peptidoglycan hydrolysis.

Metabolism and biochemistry *Neisseria* species produce oxidase and catalase, carbonic anhydrase, and nitrite reductase, but do not produce thymidine phosphorylase, nucleoside deoxyribosyl transferase, or thymidine kinase (Table BXII.β.76).

Carbohydrate metabolism *Neisseria* species do not catabolize many carbohydrates and some species (*N. cinerea*, *N. flavescens*, and *N. elongata*) are asaccharolytic. Glucose is the only carbohydrate that can be used as an energy source by *N. gonorrhoeae* and is apparently the only monosaccharide that is used by the other saccharolytic species.

Neisseria species produce acid from carbohydrates by oxidation, not fermentation. Because these species are oxidative and produce less acid from carbohydrates such as glucose, maltose, fructose, and sucrose than do fermentative organisms, and because they also produce ammonia from peptones (which may neutralize acid produced from carbohydrates), acid production must be determined in a medium with a low protein/carbohydrate ratio and a sensitive indicator such as phenol red (Knapp, 1988).

Phosphoglucose isomerase (Pgi) in *E. coli* is a dimeric enzyme that catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate in glycolysis (Froman et al., 1989; Fraenkel, 1992). In *E. coli* there is a single *pgi* gene, and Pgi appears to be dispensable because strains lacking it are also able to grow on glucose minimal media, apparently utilizing glucose primarily by the pentose phosphate shunt (Fraenkel, 1992). Expression analysis in starch gel electrophoresis and isoenzyme analysis revealed the presence of two distinct isoforms of Pgi in *N. meningitidis* and *N. gonorrhoeae*. Indeed, Southern blot analysis of *N. meningitidis* and *N. gonorrhoeae* chromosomal DNAs and the complete genome sequences confirmed that there are two complete *pgi* genes on different parts of the chromosomes in pathogenic *Neisseria* (Tønjum et al., 1994). This gene duplication may be significant since Pgi expression in other bacteria has been implicated in pathogenicity (Tung and Kuo, 1999).

Tricarboxylic acid cycle *Neisseria* species have a functional tricarboxylic acid cycle (Holten, 1975). All tricarboxylic acid cycle enzymes, except a soluble pyridine nucleotide-dependent malate dehydrogenase, have been detected in *N. gonorrhoeae* and *N. meningitidis* (Jyssum, 1960). Instead of the pyridine nucleotide-dependent malate dehydrogenase, *N. gonorrhoeae* and *N. meningitidis* possess a flavine adenine dinucleotide (FAD)-dependent malate oxidase (Holten, 1976b). A similar enzyme is present in the other *Neisseria* spp. (Holten, 1976b) in spite of the observation that these species also have a pyridine nucleotide-dependent malate dehydrogenase (Holten and Jyssum, 1974).

N. gonorrhoeae lacks a glyoxylate bypass (Holten, 1976b). With the exception of *N. elongata*, *Neisseria* spp. do not oxidize acetate in the absence of other substrates, providing additional evidence for the absence of a glyoxylate bypass (Holten, 1976b). Indirect evidence of acetate oxidation in the absence of other substrates suggests that *N. elongata* may have a glyoxylate bypass (Holten, 1976b, 1977).

Amino acid metabolism The biosynthesis of amino acids by *Neisseria* spp. appears to occur by pathways similar to those in other microorganisms. *Neisseria* species vary widely with respect to their amino acid requirements. In general, the nonpathogenic *Neisseria* species are able to grow in a defined medium containing one to five amino acids (McDonald and Johnson, 1975), while the amino acid requirements of the pathogenic species are more complex (Catlin, 1973). *N. gonorrhoeae* exhibits an absolute requirement for cysteine (or cystine) (Catlin, 1973). Strains of *N. gonorrhoeae* often require one or more amino acids in addition to cysteine for growth in a chemically defined medium (Janik et al., 1976). Amino acid requirements have been used to differentiate among isolates (auxotyping) for epidemiologic purposes (Carifo and Catlin, 1973) or for identification by genetic transformation by returning auxotrophic strains to phototrophy (Juni and Heym, 1980). Auxotyping data have also demonstrated that the requirement for certain amino acid biosynthesis in *N. gonorrhoeae* is associated with spontaneous mutations in the genes encoding enzymes involved in the biosynthesis of amino acids (Lerner et al., 1980; Shinners and Catlin, 1982).

Amino acids can be used as energy and carbon sources by many *Neisseria* spp. via their oxidation by the tricarboxylic acid cycle (Holten, 1976a). Glutamate, proline, and to a lesser extent aspartate are the preferred amino acids (McDonald and Johnson, 1975; Pillon et al., 1982). Glutamate dehydrogenase is a key enzyme in the catabolism of glutamate and proline, and all *Neisseria* species contain two forms of glutamate dehydrogenase (Holten, 1973; Holten and Jyssum, 1973). The NAD-dependent enzyme serves a catabolic function, and the NADP-dependent enzyme serves an anabolic function, depending on the needs of the cell (Holten, 1973).

Electron transport *Neisseria* spp. characteristically have high cytochrome *c* oxidase activity (Jurtshuk and Milligan, 1974). The oxidation of tetramethyl-*p*-phenylendiamine by this cytochrome provides the basis for the oxidase test used in the identification of members of the genus *Neisseria*.

Proteolytic enzymes The degradation of proteins or peptides requires specific enzymes. *Neisseria* species possess aminopeptidases that are capable of hydrolyzing L-amino-acid-β-naphthylamide derivatives of various amino acids (D'Amato et al., 1978). *N. meningitidis* can be distinguished from *N. gonorrhoeae* by the presence of *N*-γ-glutamyl aminopeptidase (D'Amato et al., 1978). However, some of the nonpathogenic species (*N. mucosa*, *N. sicca*, and all biovars of *N. subflava*) also produce *N*-γ-glutamyl aminopeptidase (Riou et al., 1982).

N. gonorrhoeae and *N. meningitidis* express a highly specific IgA protease that cleaves human IgA of the IgA1 subclass (Plaut et al., 1975; Koomey and Falkow, 1984). This enzyme is an important virulence factor in that its production promotes bacterial immune evasion. Each isolate of *N. gonorrhoeae* and *N. meningitidis* produces only one of two distinct types of IgA protease (Mulks et al., 1980). The two types of IgA protease can be distinguished by their specificity for two different peptide bonds in the hinge region of human IgA1 (Mulks and Knapp, 1987). The type I protease cleaves the proline-serine bond at position 237/238 in the IgA1 hinge region (Mulks et al., 1980), while the type 2 protease cleaves the proline-threonine peptide bond at position 235–236 (Plaut et al., 1975). The IgA protease is an extracellular enzyme that is secreted throughout exponential growth (Pohlner et al., 1987). The gene encoding IgA protease in *N. gonorrhoeae* was cloned, inactivated, and reintroduced into *N. gonorrhoeae* to

obtain an IgA protease negative mutant (Koomey et al., 1982). Gonococcal IgA protease has also been suggested to play a role in adherence and invasion processes (Lorenzen et al., 1999).

Periplasmic proteins The gonococcal periplasm is very similar to that of *E. coli* (Judd and Porcella, 1993). Several proteinases that are disulfide oxidoreductases, termed DsbA, are located in the periplasmic space (Jose et al., 1996). These autotransporters are subjected to secretion across the inner membrane in a Sec-dependent manner followed by self-directed passage and release through the outer membrane (Maurer et al., 1997). The periplasmic meningococcal superoxide dismutase C catalyzes the conversion of the superoxide radical anion to hydrogen peroxide, preventing the production of toxic hydroxyl free radicals (Wilks et al., 1998).

Iron The free iron concentration in the human and other eucaryotic hosts is extremely low and cannot support sustained microbial growth. Therefore, *Neisseria* species rely on mechanisms for obtaining growth-essential iron from their host. The pathogenic neisseriae are capable of satisfying their iron requirements with human iron-binding proteins such as transferrin and lactoferrin (see iron-regulated outer membrane proteins).

Oxygen requirements *Neisseria* species are generally considered to consist of aerobic and facultatively anaerobic organisms. *N. gonorrhoeae*, however, has been isolated from body sites where anaerobes usually are found, suggesting that *N. gonorrhoeae* can grow under reduced oxygen tension. *N. gonorrhoeae* will also grow *in vitro* under oxygen tension suitable for anaerobes (Kellogg et al., 1983). Nitrite is toxic to *N. gonorrhoeae*, so that the reduction of nitrite is only observed at low concentrations (Knapp and Clark, 1984). The addition of subtoxic concentrations of nitrite to the medium enables gonococci to grow under anaerobic conditions to a level comparable to that observed aerobically (Knapp and Clark, 1984). Gonococcal nitrate reductase is a constitutive enzyme that is synthesized under aerobic conditions in the absence of nitrite (Knapp and Clark, 1984). Since nitrite is present in biological fluids, the ability to grow aerobically or anaerobically by anaerobic nitrite respiration may be one of the factors responsible for the diversity of body sites from which gonococci can be isolated. Nitrite is reduced by all *Neisseria* spp. isolated from humans, with the possible exception of some serogroups of *N. meningitidis*, and some strains of *N. lactamica*, *N. cinerea*, and *N. polysaccharea* (Morse and Knapp, 1987).

N. gonorrhoeae grown *in vitro* under anaerobic conditions expresses several new outer membrane proteins (Clark et al., 1987). The presence of antibodies in serum samples from patients with gonorrhea that react with one or more of the anaerobically induced proteins suggests that gonococci grow anaerobically *in vivo* (Clark et al., 1988).

Carbon dioxide requirements *N. gonorrhoeae* and *N. meningitidis* both require an increased CO_2 tension for isolation from clinical specimens (Tuttle and Scherp, 1952; Griffin and Racker, 1956). Gaseous CO_2 can be replaced by the addition of bicarbonate to the medium (Talley and Baugh, 1975). Little is known about the CO_2 requirements of the nonpathogenic *Neisseria* spp. The enzymes known to be involved in the assimilation of CO_2 by *Neisseria* spp. are carbonic anhydrase, which appears to be located in the cytoplasmic membrane (MacLeod and DeVoe, 1981), and phosphenolpyruvate carboxylase (Jyssum and Jyssum, 1962; Holten and Jyssum, 1974).

Genetics

Transformation Transformation is the binding, uptake, and chromosomal integration of naked DNA. Many neisserial species are constitutively competent throughout their entire life cycle (Table BXII.β.77). DNA uptake in *Neisseria* spp. is dependent on the presence of a 10-basepair specific DNA uptake sequence (DUS, 5′-GCCGTCTGAA) (Goodman and Scocca, 1988). Genome sequencing shows that the pathogenic *Neisseria* contains approximately 1900 copies of the DNA uptake sequence (Parkhill et al., 2000; Tettelin et al., 2000) and that this sequence is found very infrequently in the genomes of other organisms. Some reports have claimed that most DUSs are located as inverted repeats downstream of open reading frames throughout the genome, serving as putative transcriptional regulators (Smith et al., 1999a). However, it has recently been shown that many of the genes engaged in genome maintenance harbor DUSs within their open reading frames. Genome-wide analysis of DUS representation in the coding sequences of *N. meningitidis* has indeed demonstrated that the group of genes engaged in DNA repair, recombination, restriction–modification, and replication exhibited the highest rate of DUSs among all designated gene groups (Davidsen and Tønjum, personal communication). A genome-wide overrepresentation of the *H. influenzae*-specific DUS was also found in the genome maintenance genes of this bacterial species. The biased distribution of the respective DUS within sequences encoding genome maintenance genes of the phylogenetically divergent *N. meningitidis* and *H. influenzae* is evidence for fundamental evolutionary prioritization and conservation. However, the complete role of DUS in this context is yet to be unraveled.

The expression of natural competence may vary since it is associated with type IV pilus expression in most species (Bøvre and Frøholm, 1971, 1972; Swanson et al., 1971; Frøholm et al., 1973). Therefore, it is often critical to perform selective culture passages to maintain pilus expression; nonselective subcultures should be minimized to avoid loss of pilus expression and thus a loss of competence. The transformation yield, however, is higher in early phases of growth, before autolysis of bacteria occurs in the culture and causes inhibition of transformation by

TABLE BXII.β.77. List of species of *Neisseria* and *Neisseriaceae* known to have naturally competent isolates

Species/Subspecies	References[a]
Neisseria elongata subsp. *elongata*	Bøvre and Holten (1970)
Neisseria elongata subsp. *glycolytica*	Bøvre et al. (1977)
Neisseria flava	Catlin and Cunningham (1961)
Neisseria flavescens	Catlin and Cunningham (1961)
Neisseria gonorrhoeae	Sparling (1966)
Neisseria lactamica	Hoke and Vedros (1982c)
Neisseria meningitidis	Catlin (1960); Jyssum and Lie (1965)
Neisseria mucosa	Bøvre and Hagen, unpublished
Neisseria perflava	Catlin and Cunningham (1961)
Neisseria sicca	Catlin and Cunningham (1961)
Neisseria subflava	Catlin and Cunningham (1961)
Neisseria weaveri	Bøvre and Hagen, unpublished
Eikenella corrodens	Tønjum et al. (1985)
Kingella denitrificans	Weir and Marrs (1992)
Kingella kingae	Henriksen and Bøvre (1968b, 1976)

[a]Additional references that relate to the taxonomic application and consequences of genetic transformation with recipients of these species/subspecies: Henriksen and Bøvre (1968b), Bøvre and Frøholm (1972), Juni (1972, 1990), Henriksen (1976), Bøvre (1979, 1980); Bøvre and Hagen, 1981), Tønjum et al. (1995b).

exogenous DNA due to saturation of DNA binding sites with residual autologous DNA.

Many reports strongly suggest that transformation is the genetic mechanism that contributes the most to the large amount of horizontal gene transfer taking place in the *Neisseria* (Feil et al., 1996; Koomey, 1998). Transformation takes place most often between closely related species, but also between more distantly related ones, contributing to species diversity and fitness for survival. Furthermore, transformation represents an invaluable tool in the study of specific determinants by virtue of the ease with which strains carrying defined mutations in chromosomal genes can be constructed.

Competence for genetic transformation provides a unique tool for measuring genetic distances for classification and identification purposes, since the relative efficiency of transformation depends on the degree of DNA homology as well as on DNA uptake sequences (Albritton et al., 1986). The widespread occurrence of natural competence for genetic transformation among *Neisseria* spp. has enabled the use of this method for critical classification schemes and strain identification (see also chapter on the *Moraxellaceae*) (Bøvre, 1965, 1980; Bøvre et al., 1977). In the 1950s, Henriksen made observations causing subdivision of bacterial groups into species of *Neisseria, Moraxella*, and other genera (Henriksen, 1952). These early studies demonstrated that it was possible to show relatedness of bacterial strains because of the ability of their DNAs to undergo genetic recombination, provided that at least one of these strains was competent for genetic transformation (Henriksen, 1952; Leidy et al., 1956; Catlin and Cunningham, 1961; Bøvre, 1964). This procedure involved quantitative transformation of streptomycin-resistance markers and has later been applied in many neisserial species (Table BXII.β.77). Juni and co-workers developed another useful and easy-to-perform transformation assay for distinction of species (Juni and Janik, 1969; Juni, 1972, 1990). This technique involved transformation of auxotrophic bacterial strains to prototrophy, using crude lysates of bacterial cells as transforming DNA. The use of lysates as transforming DNA greatly facilitates the practical application of this method. To establish nutritional transformation assays it is necessary to isolate nutritionally defective mutants of the competent strain, and also to design a minimal or defined medium on which transformants of the nutritionally deficient mutants can grow and thus demonstrate that transformation has taken place. For example, *N. gonorrhoeae* strains grow on a simple sodium lactate–mineral medium with ammonium chloride as the nitrogen source, no growth factors being required (Juni, 1974). Metabolic markers in transformation can give an increased opportunity for discrimination between closely related strains. However, this approach may in some cases be of reduced utility due to a relatively higher frequency of divergence occurring in these genes.

Conjugation Several neisserial conjugative plasmids have been described (see Plasmids and Antibiotic susceptibility).

Recombination The plasticity of the neisserial genomes for large and small genome rearrangements demands highly facilitated DNA recombination and repair systems. High-frequency recombination events may be RecA-dependent, such as gene conversion and homologous recombination, or RecA-independent, such as replicative DNA slippage. Indeed, a number of recombination genes have been identified in *Neisseria* (Carrick et al., 1998; Salvatore et al., 2002; Skaar et al., 2002). However, an in-

ducible DNA repair and recombination response (SOS response) has not been identified in *Neisseria* (Black et al., 1998).

Consequences of natural transformation, recombination, and spontaneous mutation on phylogenetic sequence analyses of* Neisseria *species The pattern of nucleotide sequence variation within genes shows that species within the genus *Neisseria* can be assigned to five phylogenetic groups, but that sequence divergence within *N. meningitidis* and closely related species is inconsistent with a bifurcating tree-like phylogeny and is better represented by an interconnected network (Smith et al., 1999a). New data indicate that although the human commensal *Neisseria* species can be separated into discrete groups of related species, the relationships both within and among these groups, including those reconstructed using 16S rDNA sequences, have been distorted by interspecies recombination events (Smith et al., 1999a). The sequence data indicate that there has been a history of interspecies recombination within selected genes of the human *Neisseria* species, which has obscured the phylogenetic relationships between the species (Feil et al., 1996). The neisseriae are therefore polyphyletic species, most probably due to the frequent DNA exchange and recombination events, both intragenomic and subsequent to transformation, taking place in these species. One consequence of the high spontaneous mutation and recombination rates detected and horizontal gene transfer is that the bifurcating phylogeny that can be inferred for other genetic bacterial entities are not valid for genus *Neisseria*, except for very closely related strains reflecting short-term genetic distance and exchange.

Among the neisseriae, the limitations of 16S rDNA based phylogeny are demonstrated due to 1) the high level of horizontal gene transfer, 2) high frequency and variability of mutation rates, as well as 3) high frequencies of gene duplication (and extinction) occurring in bacteria with high numbers of repetitive genetic elements of various kinds, such as those demonstrated by the genome sequences of the pathogenic *Neisseria* (Parkhill et al., 2000; Tettelin et al., 2000). Any phylogeny created depends on the gene chosen for analysis, and no deep evolutionary tree can be determined for the neisseriae due to the continuously high spontaneous mutation rate and recombination including incorporation of DNA from other species. If an analysis is performed that does not force the data into a tree-like phylogeny (split decomposition), then a network phylogeny can be obtained with all genes examined to date, although the precise network will differ from gene to gene.

Genomics The complete genome sequences of meningococcal isolates of both *N. meningitidis* serogroup A Z2491 and *N. meningitidis* serogroup B strain MC58 have been published, and the genomes are 2.2 and 2.3 Mbp in size, respectively (Parkhill et al., 2000; Tettelin et al., 2000). These genomes have a little over 2000 open reading frames, with 83% of each genome being coding sequence, which is the lowest percentage coding sequence among the intact bacteria so far sequenced. The mol% G + C is variable and some low mol% G + C regions are associated with open reading frames predicted to code for surface structures. Several partial genes and pseudogenes have been identified that represent the C-terminal portion of upstream genes; these may allow variation by the use of alternate C-termini. Approximately 1900 copies of the *Neisseria* uptake sequence are present throughout the genome. A comparison of the two genome sequences reveal 91.2% similarity with one inversion of

955 kb being the major difference. However, two inversion events have occurred, both of which center around the origin of replication. Repeat arrays are present near the points of inversion. A related bacteriophage has been identified in each genome, although in different positions. Three major islands of horizontal DNA transfer have been identified in the genomes; most of these contain genes encoding proteins involved in pathogenicity. However, the most notable feature of these genomes is the presence of many hundreds of repetitive elements, ranging from short repeats, positioned either singly or in large multiple arrays, to insertion sequences and gene duplications of 1 kb or more. Many of these repeats appear to be involved in genome fluidity and antigenic variation. *N. meningitidis* contains more genes that undergo phase variation than any pathogen studied to date, a mechanism that controls their expression profiles and contributes to the evasion of the host immune system.

The genome sequence of *N. meningitidis* serogroup C strain FAM18 and *N. gonorrhoeae* strain F1090 are almost completed. The genome of *N. gonorrhoeae* is approximately 2.1 Mbp with a prediction of around 2000 open reading frames and 2000 uptake sequences. Approximately 30% of the gonococcal genome is repetitive DNA with 6000 repeats less than 2 kb in size. Comparisons show that it has approximately 95% conservation with the *N. meningitidis* isolate Z2491 with one large rearrangement. Of the two isolates sequenced to date, the serogroup A Z2491 isolate was more similar in gene order to the sequenced gonococcal isolate than it was to the MC58 variant sequenced.

Plasmids and transposons Plasmids in *Neisseria* spp. have attracted considerable attention because of their potential role in virulence and association with antibiotic resistance. Beta-lactamase plasmids have been identified in *N. gonorrhoeae* and *N. meningitidis* and have been associated with resistance to penicillin and other beta-lactam antibiotics (Roberts, 1989). Several beta-lactamase-conferring plasmids have been described; these nonconjugative plasmids range in size from 2.9 to 4.4 mDa and share considerable homology with each other (Dillon and Young, 1989; Pagotto et al., 2000; Pagotto and Dillon, 2001).

Two types of larger nonconjugative plasmids have been described in *Neisseria* spp. The 24.5-mDa plasmid carries no detectable markers for antibiotic resistance. However, it efficiently mobilizes itself and the beta-lactamase plasmids between strains of gonococci. A 25.2-mDa conjugative plasmid has been described in isolates of *N. gonorrhoeae* and *N. meningitidis* (Morse et al., 1986; Knapp et al., 1988). This plasmid carries tetracycline resistance and was formed by the transposition of the TetM determinant onto the 24.5 conjugative plasmid. The 25.2-mDa plasmid will also mobilize beta-lactamase plasmids and has an extended host range (Roberts and Knapp, 1988a, b). A group of plasmids that are genetically related to the enteric plasmid RSF1010 and range in size from 4.9 to 9.4 mDa has been described in *N. meningitidis*, *N. mucosa*, *N. subflava*, and *N. sicca* (Pintado et al., 1985; Rotger et al., 1986; Facinelli and Varaldo, 1987). Some of these plasmids confer resistance to sulfonamide alone, whereas others specify resistance to sulfonamide, streptomycin, and penicillin (Rådstrøm et al., 1992). Many *Neisseria* spp. contain cryptic plasmids, i.e., plasmids with no measurable phenotype. The 2.6-mDa plasmid from *N. gonorrhoeae* is present in auxotypes of *N. gonorrhoeae*, except those strains that require proline, citrulline, and uracil (Dillon and Pauze, 1981). Some strains of *N. meningitidis*, *N. lactamica*, *N. mucosa*, and *N. cinerea* carry plasmids that are homologous to the gonococcal 2.6-mDa

plasmids (Ison et al., 1986) and that are not homologous with this plasmid (Aalen and Gundersen, 1985; Prere et al., 1985).

Conjugal transfer of genetically modified plasmids to appropriate gonococcal recipients has been most useful for research purposes (Eisenstein et al., 1977; Kupsch et al., 1996).

Phages A nontransducing bacteriophage has been isolated for *N. subflava* biovar perflava (Stone et al., 1956; Phelps, 1967). Evidence for the existence of a prophage can be found in the analysis of the pathogenicity island of the *N. meningitidis* serogroup A strain Z2491 (Klee et al., 2000).

Bacteriocins *N. meningitidis* and *N. gonorrhoeae* may release bacteriocins during growth (Kingsbury, 1966; Flynn and McEntegart, 1972; Senff et al., 1976; Allunans and Bøvre, 1996; Allunans et al., 1998). On average, 0.8% of gonococcal strains were reported bacteriocin producers (Lawton et al., 1976). In systemic meningococcal strains and isolates from healthy carriers of Norwegian origin, the overall reported frequency varied from 1.5% to 2.8% (Andersen et al., 1987; Allunans and Bøvre, 1996). While a high frequency of bacteriocin producers (13.5%, systemic strains) coincided with the peak incidence of the ongoing epidemic in Norway in 1975, the involvement of bacteriocins in meningococcal epidemiology and disease remains unclear.

Habitats The principal habitats of those *Neisseria* spp. isolated from humans are the mucous membrane surfaces. Similarly, for domestic and experimental animals the mucosal surfaces of the oropharynx are the principal habitats. Only *N. meningitidis* and *N. gonorrhoeae* are considered to be primary pathogens of humans. Other *Neisseria* species isolated from humans have been reported to be responsible for disease, e.g., *N. mucosa* (Berger et al., 1974), *N. flavescens* (Branham, 1930), and *N. subflava* (Lewin and Hughes, 1966), but it is generally considered that these species are opportunists that rarely cause infection. The species isolated from animals other than humans have caused infections, possibly as primary pathogens (*N. iguanae*) or as opportunists, also in humans (*N. weaveri* and *N. canis*) (Hoke and Vedros, 1982b; Anderson et al., 1994). These species are generally part of the normal flora of their respective animal hosts, but may have a broader host range as indicated by the isolation of *N. mucosa* from marine mammals (Vedros et al., 1973).

N. meningitidis strains are carried as normal flora in the oro- and nasopharynx of adults and children. The prevalence of *N. meningitidis* carriage varies geographically (Holten et al., 1978; Knapp and Koumanis, 1999). It has been suggested that *N. meningitidis* may occur more frequently in adults with gonorrhea and in homosexual men. Fewer than 1% of children are colonized by *N. meningitidis* during the first 4 years of life; the carriage rate increases after this age.

N. lactamica colonizes the throats of children more frequently than adults (Holten et al., 1978). *N. lactamica* colonizes the throats of as many as 4% of infants to a peak of 21% in children 18–24 months; colonization by *N. lactamica* then declines to 2% by age 14–17 years (Holten et al., 1978; Blakebrough et al., 1982; Knapp and Koumanis, 1999). It is estimated that 59% of children have been colonized by *N. lactamica* at least once by the age of 4 years. This pattern of colonization may reflect the fact that young children may drink large volumes of milk. Among *Neisseria* species, strains of *N. lactamica* are unique in their ability to use lactose; this characteristic may enhance populations of *N. lactamica* in the throats of younger children. Unlike *N. gonorrhoeae*

and *N. meningitidis*, *N. lactamica* has not been implicated as a primary pathogen, although it may be an opportunistic pathogen. The other commensal *Neisseria* species are normal inhabitants of the oro- or nasopharynx. These species are occasionally isolated from other sites but are not considered to be normal flora of sites other than the throat.

Because strains of the commensal *Neisseria* species rarely grow on selective media used to isolate *N. meningitidis* and *N. gonorrhoeae*, the prevalence of these species must be determined on a medium that does not contain colistin, the antibiotic incorporated in neisserial selective medium to inhibit the growth of the commensal species. It therefore was not possible to accurately determine the carriage rate of commensal *Neisseria* species in the early 1900s studies (Berger, 1961a). Most studies of the prevalence of *Neisseria* spp. were performed with nonselective media, e.g., blood agar, which neither inhibited the growth of other bacterial species nor permitted differentiation between colonies of *Neisseria* and related species. Thus, it is probable that the carriage rates of some species (*N. cinerea*, *N. polysaccharea*, and *N. lactamica*) were underestimated because these species occur in relatively small numbers and were probably overgrown by either non-neisserial species or the sucrose-positive *Neisseria* species (*N. subflava* biovar perflava, *N. sicca*, and *N. mucosa*). Furthermore, in these studies, strains were identified with acid detection tests that were not appropriate for the detection of the relatively small amounts of acid produced by *Neisseria* species, and additional differential tests now used to accurately identify commensal *Neisseria* species were unknown.

Some studies have been performed using selective differential media that inhibited the growth of non-neisserial species and selected for commensal *Neisseria* spp., by differentiating either between the asaccharolytic species or among several different groups of species.

The commensal *Neisseria* species have been determined to colonize the throats of adults as follows: *N. sicca*, 45%; *N. perflava*, 40%; and *N. subflava*–*N. flava*, 11% (Stechmann and Berger, 1964). It must be remembered that because nitrate reduction was not used as a differential test for the identification of *Neisseria* species at that time, strains of *N. mucosa* were not recognized by these authors. Although not verifiable, it is reasonable to assume that *N. mucosa* strains were present in the throats of these individuals. Thus, in this study, strains of *N. mucosa* were identified as either *N. sicca* or *N. perflava*, and the prevalences of these latter species were overestimated by the inclusion of strains of *N. mucosa* in their numbers (Berger and Miersch, 1970). Using a selective medium at a later time, Berger found that asaccharolytic strains, i.e., strains that do not produce detectable acid from glucose, maltose, sucrose, or fructose, accounted for 15% of all neisserial isolates, but, because nitrate reduction was not used to differentiate between *N. cinerea* and *M. catarrhalis* until a few years later, the relative colonization rates of these individual species were not given.

Pathogenicity Among the *Neisseria* and related species, only *N. gonorrhoeae* is always considered to be pathogenic and cause disease; *N. gonorrhoeae* is not considered to be normal flora under any circumstances. *N. gonorrhoeae* strains may infect the mucosal surfaces of urogenital sites (cervix, urethra, rectum) and the oro- and nasopharynx (throat), causing symptomatic or asymptomatic infections. Gonococcal infections of the urogenital sites are more frequently symptomatic than asymptomatic; however, asympto-

matic infections may occur. Gonococcal infections of the oro- and nasopharynx and the rectum may be asymptomatic more frequently than symptomatic. The prevalence of gonorrhea varies geographically (Lind, 1990; van der Heyden et al., 2000).

N. meningitidis causes epidemic meningitis in many parts of the world such as sub-Saharan Africa. Certain types of *N. meningitidis* are usually associated with meningitis. Of a total of 13 serogroups of *N. meningitidis*, strains belonging to the serogroups A, B, C, and W-135 have most frequently been associated with epidemics. Group A strains have been associated with most epidemics, whereas group B, C, W-135, and Y strains have caused sporadic epidemics. Strains of *N. meningitidis* may be carried as normal flora in the throat or nasopharynx. Between 3% and 30% of healthy persons in nonepidemic geographic areas may be asymptomatic carriers of *N. meningitidis*, i.e., meningococci have colonized their throats without causing disease. The carrier state may persist for many months. *N. meningitidis* may be considered as part of the oral normal flora particularly in crowded human settings, such as child care facilities and military camps. *N. meningitidis* is a major cause of bacterial meningitis and septicemia worldwide. From its commensal state in the pharyngeal mucous membrane, *N. meningitidis* may opportunistically disseminate to the bloodstream and, in the absence of bactericidal serum activity, cause sudden onset of disease. Systemic meningococcal disease affects primarily small children and adolescents, often leading to neurological sequelae or a fatal outcome. Despite the unique disease manifestations associated with *N. meningitidis* and *N. gonorrhoeae*, many of their basic strategies for successful colonization of their exclusive human hosts are highly conserved.

The ability of *N. meningitidis* to inflict damage on its host is correlated with adherence to mucosal epithelial cells in the nasopharynx and further invasion of subepithleial tissues and blood vessels (Nassif, 1999; Hardy et al., 2000). The most important virulence factors contributing to disseminated disease are pili, IgA1 protease, LOS, outer membrane proteins, and capsule polysaccharides (Poolman et al., 1995). *N. meningitidis* releases large amounts of the potent LOS through blebs or lysis. Meningococcal LOS exerts its impact on pathogenesis by facilitating close attachment to and inducing a potent cytokine response in host cells, mainly through TNF-α and interleukin-1 (Brandtzæg, 1995).

N. gonorrhoeae and *N. meningitidis* can cause conjunctivitis, and epidemics of gonococcal ophthalmia occur in developing countries (Koch, 1883; Myerhoff, 1911; Maxwell-Lyons and Amies, 1949). "Gonococcal" eye infection was common in Egypt even after the introduction of antimicrobial agents (Maxwell-Lyons and Amies, 1949). For example, in one study, conjunctival scrapings from children with trachoma and conjunctivitis contained intracellular diplococci. The Gram-negative bacterial strains isolated resembled *N. gonorrhoeae* in growth characteristics and sugar utilization patterns but could not be typed as gonococci. The colony morphology resembled that of meningococci more than that of gonococci. The DNA of the Egyptian isolates was cleaved by the restriction enzyme *Hae*III, like that of *N. meningitidis* but not like that of *N. gonorrhoeae*. The frequency of transformation of a temperature-sensitive mutant of *N. gonorrhoeae* to the ability to grow at the non-permissive temperature was 5–10 fold lower when DNA from two of the Egyptian isolates or from two *N. meningitidis* strains was used for transformation than when DNA

from two *N. gonorrhoeae* strains was used (Mazloum et al., 1986). One of the Egyptian isolates exhibited 68–73% DNA relatedness to *N. gonorrhoeae* DNA and 57–63% DNA relatedness to *N. meningitidis* DNA. The Egyptian isolates were therefore thought to be a variant of *N. gonorrhoeae*, which Mazloum et al. (1986) termed "*N. gonorrhoeae* subsp. *kochii*". 16S rDNA analysis confirmed "*N. gonorrhoeae* subsp. *kochii*" as an intermediate between *N. gonorrhoeae* and *N. meningitidis*.

Other *Neisseria* species are considered to be commensals; they colonize the host without causing disease. Strains of these species are normal flora in the throat and appear to be opportunistic pathogens; they may cause infections although they are not routinely associated with specific types of infections or infections of specific sites. Most *Neisseria* species have been isolated occasionally from blood, cerebrospinal fluid, abscesses, etc., but no consistent association between any species and syndrome has been established that would warrant designating any of these species as pathogens. Some infections caused by commensal *Neisseria* species have occurred in persons who have deficient immune systems and who therefore may be predisposed to infections with organisms that would not normally cause disease.

Antibiotic susceptibility Neisserial species are naturally susceptible to most antibiotics active against Gram-negative bacteria. The antibiotic susceptibilities are monitored through disk diffusion or agar dilution methods. However, antibiotic resistance is now widespread among the pathogenic neisseriae, more so in *N. gonorrhoeae* than in *N. meningitidis*, and occurs as both chromosomally mediated resistance to a variety of agents and plasmid-mediated resistance to penicillins (penicillinase/beta-lactamase producing strains) and to tetracycline (Dillon et al., 1983; Mendelman et al., 1988; Rice and Knapp, 1994). Penicillin resistance due to changes in the genes encoding PBPs is acquired through horizontal gene transfer by transformation (Spratt et al., 1992). Penicillin insensitivity in neisserial species is associated with changes in penicillin-binding protein PBP-2 occurring by horizontal gene transfer (Spratt, 1988; Spratt et al., 1989; Maggs et al., 1998). Resistance to fluoroquinolones in *N. gonorrhoeae* has already emerged and resistance to narrow-spectrum cephalosporins in *N. meningitidis* and *N. gonorrhoeae* is emerging (Fox and Knapp, 1999). Spectinomycin resistance in *N. gonorrhoeae*, due to point mutations in the gene encoding the ribosomal protein S12, and chloramphenicol-resistant *N. meningitidis* strains are increasing problems (Galimand, 1999, 2000). Sulfonamide resistance due to changes in the gene encoding dihydropteroate (*dhps*) was acquired through transformation (Rådstrøm et al., 1992). Comprehensive antibiotic susceptibility data are not available for commensal *Neisseria* spp. Generally, the commensal *Neisseria* spp. have been reported to be susceptible at least *in vitro* to penicillin, ampicillin, and tetracycline, although some strains may possess the TetM determinant. Strains of *N. cinerea*, however, appear to exhibit uniformly decreased susceptibility or resistance to erythromycin when tested by procedures for *N. gonorrhoeae* (Knapp and Koumanis, 1999).

Sulfonamide resistance is predominant in the *N. meningitidis* ET-5 complex, a distinctive group of genetically closely related clones that has been responsible for an epidemic of meningococcal disease in Norway since the mid-1970s. Clones of the *N. meningitidis* ET-5 complex have been identified as the causative agents of recent outbreaks and epidemics in many parts of the world. Analysis of sulfonamide susceptibility of isolates of the ET-5 complex from various geographic sources showed that there was no difference in resistance according to geographic source of the isolates, demonstrating that sulfonamide resistance is an essentially invariant property of clones of the ET-5 complex (Caugant et al., 1989).

Antigens and vaccines Vaccines directed against the polysaccharide capsule are available for four of the five pathogenic meningococcal serogroups (A, C, Y, W), but these vaccines offer only short-duration protection, may induce tolerance after repeated immunization, and are ineffective in infants. New conjugate polysaccharide vaccines against serogroups A, C, W-135, and Y are now available. The meningococcal C glycoconjugate vaccine currently available is approximately 70% effective for 15–17 year olds. The challenge remains in finding an effective vaccine against meningococci that express the serogroup B polysaccharide, as the sialic acid parts of this capsule mimic carbohydrates found in human fetal nervous tissue and other tissues (Bøvre, 1980). This problem is exacerbated by the continual variation of meningococcal protein antigens generated by both mutation and recombination. It is likely that vaccines directed against these molecules will become less effective with the passage of time.

Antigenic variation of surface components such as fimbriae, Opa, and LPS precludes their being relevant vaccine candidates. Other noncapsular vaccine candidates are being sought, but many are variable and give weak protection. The descending order of preference for a protein vaccine candidate employed by some manufacturers is: 1) secreted, 2) outer membrane, 3) lipoprotein, 4) periplasmic, 5) integral membrane. The genome projects have opened up new approaches in the search for new vaccine candidates. The entire genome sequence of a virulent serogroup B strain (MC58) was used to identify vaccine candidates. A total of 350 candidate antigens were expressed in *E. coli*, purified, and used to immunize mice. The sera allowed the identification of proteins that are surface exposed, that are conserved in sequence across a range of strains, and that induce a bactericidal antibody response, a property known to correlate with vaccine efficacy in humans. Among the candidates identified were four lipoproteins, two outer membrane proteins, a membrane protein, murein lytic transglycolase, HtrA serine protease, an autotransporter, and members of the iron transport system (Pizza et al., 2000). Other multiple component approaches including exploiting class I outer membrane proteins, pilin, and LOS conjugates are being pursued. Targeting the semi-conserved section of the pilus had produced some degree of cross-reactivity and an LOS conjugate had induced antibody production. In some animal models, however, the production of bactericidal antibodies may not be necessary to give protection. On the other hand, the detection of bactericidal and opsonophagocytic antibodies in mice may not guarantee that the same will be induced in the human host.

Enrichment and Isolation Procedures

Details of the isolation and processing of *Neisseria* species from humans have been provided by Morello and Bonhoff (1980) and Bartlett and Finegold (1978). In brief, specimens considered to yield pure cultures (blood, spinal fluid, urethral pus, joint fluid) are plated on prewarmed or room temperature chocolate agar (blood agar heated to 80–90°C to rupture the blood cells) and incubated at 36–37°C for a minimum of 48 h in 3–10% CO_2

atmosphere having high humidity. Specimens from body sites that may contain contaminants (cervix, oropharynx) are plated on modified Thayer–Martin medium (Martin et al., 1974) or New York City medium (Faur et al., 1973) and incubated as above. Selective media for *N. meningitidis* and *N. gonorrhoeae* contain four antimicrobial agents—vancomycin, 3–4 μg/ml; colistin, 7.5 μg/ml; trimethoprim, 5 μg/ml; and nystatin, 13.5 μg/ml—to inhibit Gram-positive bacteria, Gram-negative bacteria including commensal *Neisseria* spp., swarming *Proteus* species, and fungi, respectively. Nasopharyngeal samples may be inoculated onto chocolate and blood agar media. Generally, only morphology, Gram stain, oxidase reaction, and acidification of certain sugar media (particularly glucose, maltose, lactose, and sucrose) are used for routine identification. Immunofluorescence is often used for the gonococci and serological grouping by means of agglutination tests for the meningococci. Other *Neisseria* species from humans or animals can be isolated on Mueller–Hinton agar with or without 3% defibrinated sheep blood; once obtained in pure culture they can be identified by the characteristics listed in Table BXII.β.76.

Transport The best method for preserving viable organisms is the inoculation of specimens directly onto a nutrient medium and incubation at 35–37°C in a CO_2-enriched atmosphere immediately after collection. If specimens must be transported and it is not possible to incubate the inoculated media immediately before transport, it is more important to place the inoculated plates in a CO_2-enriched atmosphere than to incubate them at 35–37°C. Inoculated media may be held at room temperature in a CO_2-enriched atmosphere either in a candle extinction jar or commercial CO_2-generating zip-lock bags for up to 5 h without considerable loss of viability. If specimens must be transported through very high or low temperatures, the samples should be transported in a Styrofoam container. Transport of clinical samples is still often performed in semisolid non-nutritive, charcoal-containing transport media, but this method is inferior to the use of a nutrient transport system in a CO_2-enriched atmosphere. If specimens must be transported a long distance, the inoculated media should be incubated for 18–24 h before being transported, and the specimen should arrive within 48 h.

MAINTENANCE PROCEDURES

Isolates should be subcultured every 18–24 h to maintain maximum viability. A neisserial isolate will usually survive for no longer than 48 h in culture, although some isolates may survive for 72–96 h. *N. meningitidis* and *N. gonorrhoeae* are particularly sensitive to cold temperatures and autolysis (Morse and Bartenstein, 1974) and therefore need to be subcultured frequently. Frequent subculturing may lead to the loss of important factors such as fimbriae unless piliated colonies are picked, as observed in a stereomicroscope.

The best method of long-term preservation is by lyophilization in a rich broth (e.g., trypticase soy broth) containing 6% lactose, with storage of the vials at 4°C (Heckly, 1961). For long-term storage in −70°C or liquid nitrogen the bacteria are resuspended in broth containing 20% glycerol or 10–50% serum (Morello and Bonhoff, 1980). The loss of cells during freezing may be minimized by rapid freezing of the specimen in an ethanol bath containing dry ice.

Direct detection in clinical specimens Direct nonculture tests are available for detecting *N. meningitidis* and *N. gonorrhoeae*. Commercial latex agglutination tests and coagglutination tests are used to detect meningococcal polysaccharide capsular antigens in body fluids such as spinal fluid. These kits contain polyvalent antibodies against the serogroups A, C, Y, and W135 and a separate reagent for serogroup B that also detects the cross-reacting *E. coli* K1 antigen. Antigen detection should always be performed along with Gram staining and culture. Direct detection of nucleic acids can be performed by PCR and other amplification techniques (Crotchfelt et al., 1997; Backman et al., 1999; Palmer et al., 2003). Relevant target DNAs for these assays are 16S rDNA, *porA* (Ni et al., 1992), IS1106, and the gene encoding CtrA, which is involved in the excretion of capsular polysaccharide (Porritt et al., 2000). Amplifying the gene encoding SiaD can provide species and serogroup identification in the same assay (Borrow et al., 1997). A direct nucleic acid detection of 16S ribosomal RNA from *N. gonorrhoeae* with a chemiluminescence-enhanced probe assay is useful for the direct detection of gonococci in clinical samples (Granato and Franz, 1990). More recently, a ligase chain reaction-based nucleic acid amplification has provided increased sensitivity in detecting *N. gonorrhoeae* in clinical samples (Hook et al., 1997).

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Initial identification of neisseriae is based on observing Gram-negative diplococci (except for *N. elongata* and *N. weaveri* cells, which are short rods) taken from the colonies that are oxidase positive when tested with tetramethyl-*p*-phenylendiamine (Kovàcs, 1956). Another routine test used in initial identification is the production of acid from glucose, maltose, lactose, and sucrose, either by inoculation of cystine trypticase agar supplemented with 1% sugar or by measuring preformed enzymes in a rapid sugar fermentation tests (Table BXII.β.76) (Knapp, 1988). *N. lactamica* is the only neisserial species that possesses a typical β-galactosidase that is produced constitutively and will hydrolyze the chromogenic substrate *o*-nitro-phenyl-β-D-galacto-pyranoside (ONPG). Rapid methods, such as RapidNE or Quad-FERM (Bio-Merieux, Vitek, France), permit the detection of acid from neisseriae within 4 h. However, since many of the reactions in different neisserial species are identical, other phenotypic tests are needed for rapid, presumptive identification. All *Neisseria* species except *N. elongata* are catalase positive when tested with 3% hydrogen peroxide and observing the prompt evolution of bubbles of gas. The superoxol test is analogous to the catalase test, but is performed with a 30% hydrogen peroxide solution (Arko and Odugbemi, 1984). This reagent is most useful in differentiating between *N. gonorrhoeae* (strongly positive), *N. cinerea* (weakly positive), and *Kingella denitrificans* (superoxol negative). The biochemical reactions with the least variability among the species are the nitrate/nitrite reduction test (Cowan, 1974) and the synthesis of polysaccharide from 5% sucrose. The latter test employs bacteria grown on heart infusion agar containing 5% sucrose; after incubation for 2 days the colonies are tested with Lugol's iodine solution diluted 1:4 and the immediate development of a blue color indicates a positive reaction.

The presence of carbonic acid anhydrase is assayed as described by Berger and Issi (1971) and Berger and Piotrowski (1974). The test is performed by determining the minimum inhibitory concentration (MIC) of acetezolamide for cultures grown on heart infusion agar (Difco) containing 5% bovine serum. The cultures are incubated under an air atmosphere and

also under air $+10\%$ CO_2. In general, if the MIC for an air-grown strain is approximately 32 mg/ml or lower, the strain produces carbonic anhydrase; this is confirmed by finding a much higher MIC for the strain when grown under 10% CO_2. The confirmation is especially important when the MIC is borderline (e.g., 16–62 mg/ml), as may occur, for example, with some strains of *N. elongata*.

Molecular strain typing The nature of gonococcal infection as a sexually transmitted disease, the rapid course of meningococcal disease, and the capacity of some serogroups to cause large-scale epidemics necessitate the use of sensitive, reliable, and rapid typing methods of characterizing strains. Because of the high plasticity of the neisserial genomes, the choice of typing methods is dependent on the epidemiological questions to be answered and on the population genetics of the organism under investigation. With highly clonal populations comprising independent non-recombining lineages such as gonococci and serogroup A meningococci, ribotyping, multilocus enzyme electrophoresis (MLEE), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and PCR with short primers for random arbitrary polymorphism detection (RAPD) or with other gene-based primers each provides a constant measure of the relationship among strains (Yakubu et al., 1999). A more restricted portfolio of molecular methods, such as PFGE, MLEE, and MLST, is appropriate for the investigation of strains of the less clonal *N. meningitidis* serogroup B and C from localized outbreaks.

The worldwide spread of distinct ET types of *N. meningitidis* has been defined by MLEE (Caugant et al., 1989). However, MLST (Maiden et al., 1998) has been introduced to improve the resolution of bacterial isolates and contributed to the identification and tracking of the spread of virulent or drug-resistant pathogens. In MLST the nucleotide sequences of 450–500 bp of at least seven nonselected housekeeping genes are determined, revealing all of the variation at each locus. Sequences that differ at even a single nucleotide are assigned as different alleles. The large number of alleles at each of the different loci provides the ability to distinguish billions of different allelic profiles. It is extremely unlikely that two unrelated isolates would have the same allelic profile. The relatedness of isolates is displayed as a dendrogram constructed using the matrix of pair-wise differences between their allelic profiles; the pattern of nucleotide sequence variation within genes clearly resolves the major meningococcal lineages known to be responsible for invasive meningococcal disease around the world. MLST exploits the unambiguous nature and electronic portability of nucleotide sequence data for the characterization of organisms (Maiden et al., 1998). With this method the strain associations obtained are consistent with clonal groupings previously determined by MLEE. The advantage of MLST over other molecular typing methods is that sequence data are truly portable between laboratories, permitting expanding global databases for each species to be utilized on a World Wide Web site, thus enabling the exchange of molecular typing data for global epidemiology via the Internet (Enright and Spratt, 1999).The need for a method like MLST is clearly demonstrated by *N. meningitidis*, which is constitutively competent for transformation, is highly recombinogenic, and has an increased spontaneous mutation rate, representing an unusual challenge for DNA repair mechanisms to handle genome instability. MLST analysis of more than 100 isolates of *N. meningitidis* indicates that 1) identical alleles are disseminated among genetically diverse isolates, with no evidence for linkage disequilibrium, 2) different loci give distinct and incongruent phylogenetic trees, and 3) allele sequences are incompatible with a bifurcating treelike phylogeny at all loci (Holmes et al., 1999). These observations are consistent with the hypothesis that meningococcal populations consist of organisms assembled from a common gene pool, with alleles and allele fragments spreading independently, together with the occasional importation of genetic material from other species (Feil et al., 1996, 2000, 2001). Further, they support the view that recombination is an important genetic mechanism in the generation of new meningococcal clones and alleles. Consequently, for anything other than the short-term evolution of *N. meningitidis*, some researchers state that a bifurcating treelike phylogeny is not an appropriate model (Holmes et al., 1999).

Recombinational exchanges in relatively diverse species such as *Neisseria* spp. are very likely to introduce multiple polymorphic nucleotide sites, whereas point mutation results in only a single polymorphism. MLST analysis of seven housekeeping genes in a large number of meningococcal isolates (n = 126) reveals that a single nucleotide site in a meningococcal housekeeping gene is at least 80-fold more likely to change as a result of recombination than as a result of mutation (Feil et al., 1996), This per-site recombination/mutation parameter value is estimated to be 10–50-fold for *E. coli* and approximately 50-fold for *Streptococcus pneumoniae*, another naturally competent species.

DIFFERENTIATION OF THE GENUS *NEISSERIA* FROM OTHER GENERA

The genus *Neisseria* contains mostly cocci. Distinguishing cocci from short rods by microscopic observation alone may sometimes be difficult, and a reliable test that can be applied in doubtful cases is to culture the organisms in the presence of subinhibitory levels of penicillin: rod-shaped organisms form long, stringy cells, whereas cocci retain their coccal morphology. It should be noted that *N. elongata* and *N. weaveri*, the rod-shaped species in the genus *Neisseria*, produce long cells by this method (Bøvre and Holten, 1970; Andersen et al., 1993).

Table BXII.β.75 of the chapter on the family *Neisseriaceae* lists other characteristics that differentiate the genus *Neisseria* from other members of the family. The rod-shaped species *N. elongata* and *N. weaveri* can be distinguished from acinetobacters by their oxidase positive reaction and from the rod-shaped moraxellae by their ability to reduce nitrite. They can be distinguished from kingellae by their generally larger and more opaque colonies and frequent inability to produce acid from glucose; more specifically, they differ from *K. kingae* by being nonhemolytic, from *S. indologenes* by being indole negative, and from *K. denitrificans* by failing to reduce nitrate (although some exceptional nitrate-positive *N. elongata* strains have been found).

Strains of the genus *Neisseria* can be differentiated from related genera such as *Kingella* and *Eikenella* as well as the more distantly related genera *Moraxella* and *Acinetobacter* by cellular morphology, catalase, production of acid from glucose, and nitrite reduction (Table BXII.β.78).

TAXONOMIC COMMENTS

Classification of the neisseriae into defined species is the subject of intense studies and has been in a state of continuous flux for the last four decades (see reviews by Henriksen, 1976; Bøvre and Hagen, 1981; Vedros, 1981; Tønjum et al., 1995b). The high frequency of horizontal gene transfer among the neisseriae obscures the phylogenetic relationships and species definitions in

TABLE BXII.β.78. Differentiation of the species of the genus *Neisseria* from other genera of the *Neisseriaceae* and two genera of *Moraxellaceae*[a]

Characteristic	*Neisseria*	*Kingella*	*Eikenella*	*Moraxella*	*Acinetobacter*
Cell morphology:					
Cocci	+	−	−	+	−
Rods	+[b]	+	+	+	+
Oxidase test	+	+	+	+	−
Catalase test	+	−	−	+	+
Acid from glucose	(+)	+	−	−	(+)
Nitrite reduction	+	+	+	(+)	−
True waxes present in cell wall	−	−	−	(+)	D
Mol% G + C of DNA	46–58	47–55	56–58	40–48	38–47

[a]Symbols: +, positive for the majority of strains of each species; (+), positive for all strains of the majority of species, only one strain known to be negative; D, positive and negative strains about equally represented. Data compiled from Hollis et al. (1969), Catlin (1978), Bøvre and Hagen (1981), Hoke and Vedros (1982a).

[b]Only two species, *N. elongata* and *N. weaveri*, consist of rods.

this genus. The degree of genetic relatedness between *N. gonorrhoeae* and *N. meningitidis* is extremely high. Kingsbury et al. (1969) found by thermal stability of hybrid DNA duplexes that the two species had at least 80% similarity in their nucleotide sequences, and the DNA–DNA hybridization studies by Hoke and Vedros (1982c) indicated a relatedness of 93%. More recently performed subtractive hybridization analysis and genome sequencing data indicate that the genome sequence similarity among the pathogenic neisseriae is higher than 96% (Tinsley and Naissif, 1996), which has been confirmed by the complete genome sequences (Parkhill et al., 2000; Tettelin et al., 2000). On purely genetic grounds, the two species therefore should be considered as subspecies of a single species. Yet, *N. meningitidis* and *N. gonorrhoeae* cause distinctly different kinds of clinical infections, and from a practical viewpoint it seems desirable to continue to consider these organisms as separate species.

Lactose-positive strains of organisms resembling *N. meningitidis* were recognized as early as 1934 (Jessen, 1934), but were largely ignored until the report by Hollis et al. (1969). *N. lactamica* showed a close relationship to *N. meningitidis* by transformation studies and to the other "true neisseriae" by DNA–DNA hybridization, cellular fatty acid composition, and mol% G + C values (Bøvre et al., 1972; Hoke and Vedros, 1982a, c).

The close relationships between all of the "true neisseriae" have been demonstrated consistently by a variety of techniques (see Bøvre, 1980; Bøvre and Hagen, 1981; Hoke and Vedros, 1982a, c). Of particular interest have been the similarities among *N. flava*, *N. perflava*, and *N. subflava*. It is difficult to differentiate these three species by cultural and biochemical reactions, and in the eighth edition of *Bergey's Manual* they were incorporated in the single species *N. subflava* (Reyn, 1974). This close similarity is supported by DNA–DNA hybridization and genetic transformation studies (Hoke and Vedros, 1982a), as well as by 16S rDNA sequence analysis (Fig. BXII.β.62). Although a high level of DNA relatedness between *N. sicca* and the *N. subflava* group has been found (Hoke and Vedros, 1982a), biochemical distinction between *N. perflava* and *N. sicca* has also been reported (Berger and Catlin, 1975). Further confusion in the classification of these chromogenic neisseriae has been added by the finding of identical lipopolysaccharides in *N. subflava* and *N. canis* (Johnson et al., 1976).

New species in genus *Neisseria* since the last edition of *Bergey's Manual of Systematic Bacteriology* (1984) are *N. polysaccharea* (Riou and Guibourdenche., 1987), *N. macacae, N. iguanae, N. weaveri,* *N. animalis,* and *N. dentiae* . The last four of these all have mammalian hosts other than humans.

Although the species *N. elongata* consists of rod-shaped cells, genetic transformation studies have shown that *N. elongata* has very high genetic affinities to the coccal species of *Neisseria* (Bøvre and Holten, 1970; Bøvre et al., 1977) and has no affinities to other genera of the family *Neisseriaceae* except a low affinity to *Kingella kingae. N. elongata* possesses carbonic anhydrase, which is characteristic of the "true neisseriae", and also possesses a fatty acid composition similar to other neisserial species (Jantzen et al., 1974; Hoke and Vedros, 1982c). The evidence shows that the rod-shaped *N. weaveri* belongs in the genus *Neisseria*, most closely related to *N. elongata, N. flavescens,* and *N. animalis.*

The taxonomic status of the previous "false neisseriae", that is the animal species *N. caviae, N. ovis,* and *N. cuniculi,* as well as *M. catarrhalis,* has been reassigned to the *Moraxellaceae* (Rossau et al., 1989; Pettersson et al., 1998b). These species were previously considered as a group within the *Neisseria* spp., because they share several phenotypic characteristics (Henriksen, 1976; Bøvre, 1984a). They are listed and described in the article on the genus *Moraxella* in this *Manual.*

FURTHER READING

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DIFFERENTIATION OF THE SPECIES OF THE GENUS *NEISSERIA*

Neisseria species are relatively inert biochemically, but those activities and characteristics that are of determinative value are shown in Table BXII.β.76.

List of species of the genus Neisseria

1. **Neisseria gonorrhoeae** (Zopf 1885) Trevisan 1885, 106[AL] (*Merismopedia gonorrhoeae* Zopf 1885, 54.)
   go.nor.rhoe′ ae. Gr. n. *gonorrhoeae* gonorrhoea; M.L. gen. n. *gonorrhoeae* of gonorrhoea.

   Common name: gonococcus.

   The biochemical characteristics are as described for the genus and as listed in Table BXII.β.76. Primary isolation is made on chocolate agar at temperatures of 35–36°C under an atmosphere containing 3–10% CO_2 and high relative humidity. Minimum growth temperature, 30°C. At 48 h, colonies are 0.6–1.0 mm in diameter, opaque, grayish white, raised, finely granular, glistening, and convex. They become mucoid with further incubation. The cocci are arranged in pairs. Primarily found in gonorrhea (purulent venereal discharges), oropharyngeal infection, anorectal infection, endometritis, conjunctivitis, and disseminated gonococcal infection. Also found in blood, the conjunctiva, petechiae, pharynx, and cerebrospinal fluid. Found only in humans.

   The mol% G + C of the DNA is: 49.5–53.3 (T_m, chromatography).

   Type strain: ATCC 19424, CCUG 26876, CIP 79.18, DSM 9188, NCTC 8375.

   GenBank accession number (16S rRNA): X07714.

   This species shows very high genetic relatedness to *N. meningitidis* (Tinsley and Nassif, 1996; Fig. BXII.β.62). "*N. kochii*" is considered to be a subspecies of *N. gonorrhoeae* and has no independent taxonomic status.

2. **Neisseria animalis** Berger 1960, 160[AL]
   an.i.mal′ is. L. n. *animal* animal; L. gen. n. *animalis* of an animal.

   Typical diplococci. Colonies are smooth, round, graywhite. Produces acid from saccharose, no acid from fructose and maltose (which is positive for *N. sicca* and *N. perflava*, the other saccharose-positive *Neisseria*) (Berger, 1962). Isolated from the throats of guinea pigs. Appears to be closely related to *N. denitrificans* (Fig. BXII.β.62).

   The mol% G + C of the DNA is: not determined.

   Type strain: ATCC 14678, CCUG 808, CIP 72.15, NCTC 10212.

   GenBank accession number (16S rRNA): AJ239288.

3. **Neisseria canis** Berger 1962, 455[AL]
   ca′ nis. L. gen. n. *canis* of the dog.

   The cells are typical diplococci, rarely tetrads. Colonies are smooth, butyrous, with a light yellowish tinge. Absorption peaks of the extracted pigment are similar to those of *N. lactamica* (Hoke and Vedros, 1982c). The cellular fatty acids are similar to those of the "true" neisseriae. Carbonic anhydrase is produced (Berger and Issi, 1971). Other characteristics are listed in Table BXII.β.76. Isolated from the throats of cats (Berger, 1962) and as an opportunist in a cat-bite wound of a human (Hoke and Vedros, 1982b).

The mol% G + C of the DNA is: 49.6 (T_m).

Type strain: ATCC 14687, CIP 103347, LMG 8383, NCTC 10296.

GenBank accession number (16S rRNA): L06170.

16S rDNA sequence analysis of the type strain suggests that this species is more closely related to genus *Kingella* than to *Neisseria* spp.

4. **Neisseria cinerea** (von Lingelsheim 1906) Murray 1939, 283[AL] (*Micrococcus cinereus* von Lingelsheim 1906, 396.)
   ci.ne′ re.a. L. fem. adj. *cinerea* gray.

   The cocci are plump and arranged in pairs or more often in scattered clusters. Colonies are small (1.0–1.5 mm in diameter), grayish white with entire edges, and slightly granular. The percentage of fatty acids having a chain length of over 16 carbon atoms is similar to that of the other neisseriae (Hoke and Vedros, 1982c). Carbonic anhydrase is produced (Berger and Issi, 1971). Other characteristics are listed in Table BXII.β.76. Found in the nasopharynx of humans. Opportunistic pathogen (e.g., newborn ocular infections) (Bourbeau et al., 1990).

   The mol% G + C of the DNA is: 49.0–50.9 (T_m, Bd).

   Type strain: ATCC 14685, CCUG 2156, CIP 73.16, DSM 4630, LMG 8380, NCTC 10294.

5. **Neisseria denitrificans** Berger 1962, 455[AL]
   de.ni.tri′ fi.cans. L. prep. *de* away from; L. n. *nitrum* soda; M.L. n. *nitrum* nitrate; M.L. v. *denitrifico* to denitrify; M.L. part. adj. *denitrificans* denitrifying.

   The percentage of fatty acids with chain length over 16 carbon atoms and the fatty acid profile are similar to those of the "true" neisseriae (Hoke and Vedros, 1982c). Carbonic anhydrase is produced (Berger and Issi, 1971). Other characteristics are as listed in Table BXII.β.76. Isolated from the throats of guinea pigs.

   The mol% G + C of the DNA is: 55.6 (T_m).

   Type strain: ATCC 14686, CCUG 2155, CIP 72.16, NCTC 10295.

   GenBank accession number (16S rRNA): L06173, M35020.

6. **Neisseria dentiae** Sneath and Barrett 1997, 915[VP] (Effective publication: Sneath and Barrett 1996, 357.)
   den′ ti.ae. M.L. fem. gen. sing. n. *dentiae* of (Dr.) Dent; named for Vija E. Dent (Mrs. Pratley) in recognition of her pioneering work on neisserias from dental plaque.

   Cells are small cocci about 1 μm in diameter, arranged mainly as diplococci with occasional tetrads, but without diplobacilli. Nonmotile. No endospores. Growth is aerobic with optimal temperature close to 35°C. No growth under anaerobic conditions. Colonies on horse blood agar are round transparent and domed, without obvious yellow pigment, about 1 mm in diameter after 24 h growth at 35°C. No hemolysis on horse blood; weak hemolysis on sheep

blood is occasionally found. Oxidase positive. Catalase positive. Indole negative. Gelatin negative. Nitrate negative. Most strains reduce nitrite. Found in dental plaques of domestic cows. Resembles *N. animalis*, *N. canis*, and *N. iguanae* phenotypically, but is distinguished from the first two by being positive for acidification of gluconate, D-glucose, and usually D-fructose, and from the third by lacking predominant tetrad arrangement and distinct α-hemolysis and by growing on nutrient agar and usually acidifying D-fructose. May have impact on dental microbiology because members of the genus *Neisseria* rapidly utilize oxygen and this may contribute to the anaerobic microenvironment found in dental plaques.

The mol% G + C of the DNA is: not determined.

Type strain: V33, SHI/3848, ATCC 700276, CIP 106968.

GenBank accession number (16S rRNA): AF487709.

16S rDNA sequence analysis shows highest homology to *N. canis* (as for the other neisseriae from animal habitats other than humans).

7. **Neisseria elongata** Bøvre and Holten 1970, 73[AL]

e.lon′ga.ta. L. fem. part. adj. *elongata* elongated, stretched out.

Rods, short and slender, ~0.5 μm in diameter, often arranged as diplobacilli or in short chains. A marked elongation effect of sublethal concentrations of penicillin occurs during growth, with formation of very long filaments. Often fimbriated. Colonies on blood agar are shiny and low convex with an entire edge, ~2–3 mm in diameter after 48 h of incubation. The colonies are semi-opaque to grayish white with a yellowish tinge due to pigment production. Older colonies may attain a diameter of 4–5 mm and often show granular spreading zones around the periphery, or the colonies become irregular in outline with spreading projections. The colony texture is usually clay-like and coherent, and the growth mass when collected is lumpy and difficult or impossible to disperse. No hemolysis occurs. Agar corrosion with a peripheral groove and a central pit is often observed under the colony. The spreading and corrosion are related to the presence of fimbriae on the cells; nonfimbriated or less fimbriated variants give rise to colonies that are often smaller, nonspreading, and noncorroding. Optimal growth temperature 33–37°C. Capable of growing weakly on blood agar under anaerobic conditions. Grows on simple peptone media. The specific growth requirements are not known.

Capsules are not formed. Nonmotile. Oxidase positive. Usually acid is not formed from glucose, but some strains may form small amounts of acid. Nitrite reducing, but usually not nitrate reducing (see below). Catalase activity is usually not detectable but may be positive or weakly positive in some strains. No liquefaction of coagulated serum or gelatin occurs. Urease negative. Phenylalanine is not produced except for weak reactions observed with some strains. Carbonic anhydrase is produced (Berger and Issi, 1971). Highly sensitive to penicillin. The species lacks true cellular waxes (Jantzen et al., 1976) and contains heptose (Jantzen et al., 1976). Other characteristics are listed in Table BXII.β.76. Isolated from the pharynx of healthy individuals and from cases of pharyngitis. Also isolated from bronchial aspirates, pus from perimandibular abscesses, blood cultures during endocarditis, and from the urinary tract. So far recorded only from human sources. Considered as a largely harmless parasite.

The mol% G + C of the DNA is: 53.0–53.5 (T_m).

Type strain: ATCC 25295, CCUG 2043, CCUG 2130 A, CIP 72.27, LMG 5124, NCTC 10660.

GenBank accession number (16S rRNA): L06171.

The cells are frequently competent in genetic transformation and can be identified by transformation. The competence is apparently associated with the fimbriated state (Bøvre and Holten, 1970; Bøvre et al., 1977). There is a distinct genetic affinity by transformation between *N. elongata* and the coccal species of the "true" neisseriae. The species also fits in with the "true" neisseriae with respect to cellular lipid and carbohydrate composition and the characteristics of glycolytic enzymes (see reviews by Bøvre, 1980; Bøvre and Hagen, 1981).

The species is genetically somewhat heterogeneous (Bøvre et al., 1972) and it is now proposed to consist of three subspecies: *Neisseria elongata* subsp. *elongata*, *Neisseria elongata* subsp. *glycolytica*, and *Neisseria elongata* subsp. *nitroreducens*.

Another subspecies, "*N. elongata* subsp. *intermedia*", was proposed by Berger and Falsen (1976). This subspecies presently has no standing in nomenclature. It is catalase positive and immunologically distinct from the type strain of the elongata. It may possibly be identical to the subsp. *glycolytica*.

a. **Neisseria elongata** *subsp.* **elongata** Bøvre and Holten 1970, 73[AL]

Oxidase positive, aerobic, nonmotile, rod-shaped bacterium. No acid production from glucose or other carbohydrates. Catalase negative. Urease, indole, and motility negative. Nitrite reducing. Differs from subsp. *glycolytica* and subsp. *nitroreducens* by showing no acidification of glucose media and being catalase negative.

The mol% G + C of the DNA is: 56 (T_m).

Type strain: ATCC 25295, CCUG 2043, CCUG 2130 A, CIP 72.27, LMG 5124, NCTC 10660.

GenBank accession number (16S rRNA): L06171.

b. **Neisseria elongata** *subsp.* **glycolytica** Henriksen and Holten 1976, 480[AL]

gly.co.ly′ti.ca. Gr. *glyko-* from Gr. *glykys* sweet; Gr. adj. *lyticus* dissolving; M.L. fem. adj. *glycolytica* meant to indicate an ability to attack glucose.

Oxidase positive, aerobic, nonmotile, rod-shaped bacterium. Nitrite reducing. Catalase positive. Urease, indole, and motility negative. No acid production from carbohydrates. Differs from elongata by being catalase positive, by causing a weak acidification of glucose media, and by forming colonies with a smooth texture. Quantitative genetic transformation data show identity reactions between subsp. *elongata* and subsp. *glycolytica*, and they are also indistinguishable in terms of fatty acid composition (Bøvre et al., 1977).

The mol% G + C of the DNA is: 56 (T_m).

Type strain: ATCC 29315, CCUG 6508, CIP 82.85, NCTC 11050.

Although subspeciation of *N. elongata* subsp. *elongata* and *N. elongata* subsp. *glycolytica* has been proposed based

on differences in glycolytic properties (Henriksen and Holten, 1976), genetic and other evidence for subspeciation are rather weak. In fact, Bøvre et al. (1977) has reported general species identification of the glycolytic strain 6171/75 ATCC 23915 with the type strain M2 = ATCC 25295 by genetic transformation. They found DNA homology of up to 86% between the glycolytic strain (6171/75) and the type strain (M2) by quantitative streptomycin resistance transformation.

c. **Neisseria elongata** *subsp.* **nitroreducens** Grant, Brenner, Steigerwalt, Hollis and Weaver 1990, 2596[VP]

nit.ro.re′du.cens. Gr. *nitroreducens* the agent that reduces nitrate.

Oxidase positive, aerobic, nonmotile, rod-shaped bacterium. Reduction of nitrate and nitrite with no gas formation. Negative reactions for catalase, urease, indole, and motility; and no acid production from carbohydrates. Differences from subsp. *elongata*: reduces nitrate and sometimes causes a weak acidification of glucose media. Differences from *N. elongata* subsp. *glycolytica*: catalase negative, reduces nitrate. Found in throat or sputum and from blood, with many of the systemic isolates being associated with endocarditis. Rarely occurring, but can cause serious human infections (Hofstad et al., 1998). The fact that it is found in association with endocarditis and other systemic diseases differentiates it from the other *N. elongata* subspecies.

The mol% G + C of the DNA is: 55–58 (T_m).

Type strain: B109, ATCC 49377, CCUG 30802, CIP 103511, NCTC 12736.

N. elongata biovar nitroreducens was previously termed CDC group M-6. By the hydroxyapatite method, DNAs from the M-6 strains showed an average of 78% relatedness to M-6 reference strain B1019 in reactions at 60°C and 73% relatedness in reactions at 75°C (Grant et al., 1990). This organism is biochemically similar to *Kingella denitrificans* and displays a cellular fatty acid profile consistent with CDC groups M-5 and EF-4 as well as with *N. elongata*. The relatively high mol% G + C content of DNA supported the establishment of this subspecies. 16S rDNA sequence analysis show high similarities among the three subspecies of *N. elongata* (Fig. BXII.β.62).

8. **Neisseria flavescens** Branham 1930, 849[AL]

fla.ves′cens. L. v. *flavus* to become golden yellow; L. part. adj. *flavescens* becoming golden yellow.

The characteristics are as described for the genus and as listed in Table BXII.β.76. Cocci in pairs and tetrads. Colonies are smooth and opaque with golden yellow pigment. Nitrate and nitrite reducing. Synthesis of polysaccharide positive (iodine test). Found in cerebrospinal fluid of patients with meningitis and in cases of septicaemia. Rare (Branham, 1930; Wertlake and Williams, 1968). Found in the pharynx of humans (rare).

The mol% G + C of the DNA is: 46.5–50.1 (T_m, Bd, chromatography).

Type strain: ATCC 13120, CCUG 345, CCUG 17913, CIP 73.15, LMG 5297, NCTC 8263.

GenBank accession number (16S rRNA): L06168.

9. **Neisseria iguanae** Barrett, Schlater, Montali and Sneath 1994b, 852[VP] (Effective publication: Barrett, Schlater, Montali and Sneath 1994a, 201.)

i.gu′an.ae. M.L. fem. gen. sing. n. *iguanae* of the iguana lizard; from Sp. fem. n. iguana.

Cells are small cocci about 0.8 μm in diameter, largely arranged as diplococci but show numerous tetrads in culture. Nonmotile. Surface colonies grown on 5% sheep or horse blood agar are nonpigmented, round, transparent, domed, about 1 mm in diameter after 72 h at 35°C with marked zones of α-hemolysis. Oxidase positive, catalase positive, urease and deoxyribonuclease negative. Weak acidity usually from glucose, sucrose, and gluconate. Strongly positive for alkaline phosphatase. Gluconate positive. Strongly positive for alkaline phosphatase 2. No endospores. Aerobic growth, no growth under anaerobic conditions. Mesophilic. Grows between 25°C and 37°C. Chemoorganotrophic. May be isolated from the oral cavity of healthy lizards. Associated with septicemia and abscesses in iguanid lizards (*Iguana iguana* and *Cyclura cornuta*).

The mol% G + C of the DNA is: 50.8–52.0 (T_m).

Type strain: ATCC 51483, NVSL 85737.

10. **Neisseria lactamica** Hollis, Wiggins and Weaver 1969, 72[AL]

lac.ta.mi′ca. L. n. *lac* milk, from *lactose* milk sugar; L. adj. *amicus* fond of; M.L. fem. adj. *lactamica* fond of lactose.

The characteristics are as described for the genus and as listed in Table BXII.β.76. Cocci arranged in pairs. Colonies are small, smooth, translucent, slightly butyrous, and often have a yellowish tinge. Primary isolation is made on chocolate agar at temperatures of 35–36°C under an atmosphere containing 3–10% CO_2 and high relative humidity. Minimum growth temperature 30°C. At 48 h, colonies are 0.6–1.0 mm in diameter, opaque, grayish white, raised, finely granular, glistening, and convex. They become mucoid with further incubation. The only *Neisseria* species that produces acid from lactose. Primarily found in the nasopharynx, most commonly found in children and young teenagers. Rarely pathogenic, but has been identified as a possible cause of meningitis.

The mol% G + C of the DNA is: 49.5–53.3 (T_m, chromatography).

Type strain: ATCC 23970, CCUG 5853, CIP 72.17, DSM 4691, NCTC 10617.

GenBank accession number (16S rRNA): AJ239286.

Genetic transformation, DNA–DNA hybridization, and 16S rDNA sequence analysis have indicated a close relationship between *N. lactamica*, *N. meningitidis*, and *N. gonorrhoeae*, but not as close as that between *N. meningitidis* and *N. gonorrhoeae* (Siddiqui and Goldberg, 1975; Hoke and Vedros, 1982a; Garborg and Tønjum, personal communication).

11. **Neisseria macacae** Vedros, Hoke, and Chun 1983, 519[VP]

ma′ca.cae. Port. n. *macaco* female monkey; N.L. fem. gen. *macacae* of a monkey, referring to the source of the isolates.

Cocci occurring in pairs with adjacent sides flattened or singly; cells are nonmotile and 0.6–1.0 μm in diameter. Colonies on Mueller–Hinton agar or nutrient agar are slightly raised, yellowish green, and glistening, have entire edges, and are about 1–1.5 mm in diameter after 17 h of incubation at 37°C in a humid atmosphere. Aerobic. Optimal

growth at 35–37°C; less growth at 30°C. Growth enhanced by 5–8% CO_2 at 30°, 35°, or 37°C. Slight or no growth at 22° and 42°C. Moderate hemolysis of horse blood agar and rabbit blood agar. Oxidase positive. Catalase positive. Nitrate is not reduced, nitrite is reduced with the production of gas. Polysaccharides are produced from sucrose, DNA is hydrolyzed, tributyrin is not hydrolyzed. Isolated from the oropharynges of rhesus monkeys.

The mol% G + C of the DNA is: 50–51 (T_m).

Type strain: M-740, ATCC 33926.

GenBank accession number (16S rRNA): L06169.

12. **Neisseria meningitidis** (Albrecht and Gohn 1901) Murray 1929, 8[AL] (*Micrococcus meningitidis* Albrecht and Gohn 1901, 498.)

me.nin.gi′ ti.dis. Gr. n. *meninx, meningis* the membrane enclosing the brain; M.L. fem. n. *meningitis, meningitidis* inflammation of the meninges.

Common name: meningococcus.

Cocci arranged in pairs. Cellular division occurs in two planes with the second division at a right angle to the first. Transient tetrads can therefore be observed in wet mounts of young, growing cultures (less than 8-h-old). Primary isolation is made on blood agar, chocolate agar, or Mueller–Hinton agar. An atmosphere containing 3–10% CO_2 and high relative humidity enhances growth. Optimal temperatures 36–37°C. Colonies vary in size depending on the medium, extent of crowding, and length of incubation; in 18–24 h they are approximately 1.0 mm in diameter. Colonies are small, round, smooth, glistening, sometimes mucoid and translucent on Mueller–Hinton agar, and are often iridescent. Due to autolysis with age, colonies become more butyrous and rubbery to the touch of an inoculating needle. Other characteristics are listed in Table BXII.β.76. Some strains are erratic in producing acid from maltose and glucose (Jyssum and Jyssum, 1968; Bøvre, 1969). Found in cerebrospinal fluid as the causative agent of cerebrospinal meningitis and in blood as the cause of septicemia (including Waterhouse–Friderichsen syndrome), lower genital tract infections (rare), and pneumonia (rare). Can be cultivated from petechiae, joints, nasopharynx, and conjunctiva, occasionally found in venereal discharges. Frequently found in a commensal state in the oro- or nasopharynx of asymptomatic carriers.

The mol% G + C of the DNA is: 50–52 (T_m, chromatography).

Type strain: M1027, ATCC 13077, CCUG 3269, CIP 73.10, DSM 10036, NCTC 10025.

N. meningitidis shows a very high genetic relatedness to *N. gonorrhoeae* by DNA–DNA hybridization (>80%) (Kingsbury et al., 1969) and by subtractive hybridization (96% similarity) (Tinsley and Nassif, 1996).

13. **Neisseria mucosa** (von Lingelsheim 1906) Véron, Thibault and Second 1959, 508[AL] (*Diplococcus mucosus* von Lingelsheim 1906, 395.)

mu.co′ sa. L. fem. adj. *mucosa* slimy.

The characteristics are as described for the genus and as listed in Table BXII.β.76. Cocci arranged in pairs. Colonies are large, mucoid, and often adherent. Most strains are nonpigmented (Berger and Miersch, 1970) or grayish to buff yellow colonies, but one strain has been described

as slightly yellow (Véron et al., 1959). Found in the nasopharynx of humans and, in one report, as part of the normal flora of the respiratory tissues in dolphins (Vedros et al., 1973). Occasionally pathogenic for humans, causing pneumonia in children (rare). Pathogenic for mice.

The mol% G + C of the DNA is: 50.5–52.0 (T_m, Bd).

Type strain: ATCC 19696, CCUG 26877, CIP 59.51, DSM 4631, NCTC 12978.

An organism called "*N. mucosa* biovar heidelbergensis" was described by Berger (1971). It differed from "*N. mucosa* biovar mucosa" in that some strains were pigmented, possessed deoxyribonuclease, and produced more gas from nitrite. "*N. mucosa* mucosa" has white and larger colonies of more dry consistence than "*N. mucosa* biovar heidelbergensis". Growth in broth results in pellet forming. "*Neisseria mucosa* biovar heidelbergensis" has smaller colonies of more butyrous consistence than "*N. mucosa* biovar mucosa". Yellow pigment on blood agar. Diffuse growth in broth. 16S rDNA sequence analysis shows significant differences between "*N. mucosa* biovar mucosa" and "*N. mucosa* biovar heidelbergensis". By 16S rDNA sequence analysis *N. mucosa* strains are most closely related to *N. sicca* and *N. subflava* biovar flava.

14. **Neisseria polysaccharea** Riou and Guibourdenche 1987, 163[VP]

pol.y.sac.cha.re.a. Gr. adj. *poly* many; Gr. n. *Saccharum* sugar; M.L. fem. adj. *polysaccharea* with many saccharides.

Unencapsulated cocci arranged in pairs or tetrads. Oxidative respiratory metabolism. Oxidase and catalase produced. All strains grow on selective medium producing small, grayish yellow colonies that are translucent and raised of about 2 mm in diameter after 24 h at 37°C. No growth occurs at 22°C. Large amounts of polysaccharide are produced on solid or in liquid medium containing 1% or 5% sucrose. Requires cystine–cysteine for growth on *Neisseria* defined medium (Catlin, 1973); in addition, some strains require arginine. Acid is produced from glucose and maltose, rarely from sucrose, but never from fructose, D-mannitol, or lactose. Tributyrin and *o*-nitrophenyl-β-D-galactopyranoside are not hydrolyzed. Nitrites are reduced, nitrates are not. Deoxyribonuclease, gelatinase, and γ-glutamyltransferase are not produced. No hemolysis is observed on horse blood agar. Produces extracellular polysaccharide and exhibits γ-glutamyltransferase activity. It is found in the nasopharynx of infants and children. No strain of this species has been recognized as a cause of human disease.

The mol% G + C of the DNA is: 52.5–54. (T_m, Bd).

Type strain: ATCC 43678, CCUG 18030, CIP 100113, NCTC 11858.

GenBank accession number (16S rRNA): L06167, AJ239289.

15. **Neisseria sicca** (von Lingelsheim 1908) Bergey, Harrison, Breed, Hammer and Huntoon 1923a, 43[AL] (*Diplococcus siccus* von Lingelsheim 1908, 476.)

sic′ ca. L. fem. adj. *sicca* dry.

The characteristics are as described for the genus and as listed in Table BXII.β.76. Cocci arranged in pairs and tetrads. Colonies are usually large (up to 3 mm), grayish white, opaque, dry, wrinkled, and adherent. Forms opaque, dry, wrinkled, adherent, colonies but this may vary with some strains. Some strains may produce a xanthophyll pig-

ment (Berger, 1961b); when extracted, this pigment shows absorption peaks similar to the pattern exhibited by the pigments of *N. perflava*, *N. flava*, and *N. mucosa* (Hoke and Vedros, 1982c). Spontaneous agglutination occurs in saline. The strain used in the report on envelope proteins is questionable (Russell et al., 1975). *N. sicca* may be serologically distinct from *N. subflava* (*N. flava*, *N. perflava*) and *N. flavescens* (Berger and Brunhoeber, 1961; Berger and Wulff, 1961). The species is serologically related to *N. mucosa* (Véron et al., 1959). Found in the nasopharynx, saliva, and sputum of humans (very common); opportunistic pathogen.

The mol% G + C of the DNA is: 49.0–51.5 (T_m, Bd, chromatography).

Type strain: ATCC 29256, CCUG 23929, CCUG 24959, CIP 103345, LMG 5290, NRL 30,016.

Genetic transformation and DNA–DNA hybridization indicate that *N. sicca* should be considered for inclusion with *N. perflava* and *N. flava* into the single species *N. subflava* (Catlin and Cunningham, 1961; Hoke and Vedros, 1982a). However, 16S rDNA sequence analysis shows closer relationship to *N. mucosa* and *N. macacae* (Pettersson and Tønjum)

16. **Neisseria subflava** (Flügge 1886) Trevisan 1889, 32[AL] (*Micrococcus subflavus* Flügge 1886, 159; *Neisseria flava* Bergey, Harrison, Breed, Hammer and Huntoon 1923a, 43.)
    sub.fla′va. L. pref. *sub* less than; L. adj. *flavus* yellow; L. fem. adj. *subflava* yellowish.

The characteristics are as described for the genus and as listed in Table BXII.β.76. Cocci arranged in pairs and tetrads. Colonies are smooth, transparent or opaque, often adherent. Grows on blood agar at 22°C. Some strains produce a yellowish pigment. Often agglutinates spontaneously in saline (Reyn, 1974). Found in secretions from the human nasopharynx and rarely in cerebrospinal fluid in cases of meningitis (Noguchi et al., 1963; Baraldes et al., 2000).

The mol% G + C of the DNA is: 48.0–51.0 (T_m, Bd, chromatography).

Type strain: ATCC 49275, CCUG 23930, CCUG 33675, CIP 103343, LMG 5313, NRL 30,017.

Neisseria subflava contains the previous species *Neisseria flava*, *Neisseria perflava*, and *Neisseria subflava*. *N. subflava* biovar flava and *N. subflava* biovar perflava produce acid from fructose, whereas *N. subflava* biovar subflava does not. *N. subflava* biovar perflava produces acid and polysaccharide from sucrose which *N. subflava* biovar flava and *N. subflava* biovar subflava do not. 16S rDNA sequence analysis shows that *N. subflava* biovar subflava and biovar perflava are considerably more closely related to each other than they are to *N. subflava* biovar flava. Inclusion of *N. sicca* into this species could be considered based on its relatedness to *N. subflava* biovar flava.

17. **Neisseria weaveri** Holmes, Costas, On, Vandamme, Falsen and Kersters 1993, 691[VP]
    wea′ver.i. M.L. gen. n. *weaveri* was named in honor of Robert E. Weaver.

The cells are broad, plump, medium-to-large, straight rods of varying length when grown on slants and plates, with a tendency to grow in chains or longer rods in broth cultures. They are nonmotile, aerobic, and non-salt requiring. Grow well between 25° and 35°C; most strains grow at 42°C. Colonies are gray-white with an entire border, flat, somewhat glistening, and smooth and variable in size. They are 1–2 mm in diameter after 24 h of incubation at 35°C and 2–4 mm after 48 h of incubation. A zone of α-hemolysis is produced on sheep blood agar plates in areas of heavy growth. The oxidase and catalase reactions are strongly positive. The bacterium does not utilize carbohydrates; it uses nitrite but not nitrate and has a weakly positive phenylalanine deaminase reaction from culture grown on sheep blood agar plates. It is found as normal oral flora in dogs and is associated with human wound infections resulting from dog bites.

The mol% G + C of the DNA is: 50.8–52.0 (T_m).

Type strain: ATCC 51410, CCUG 4007, CCUG 33675, CIP 103940, ISL775/91, LMG 5135, NCTC 12742.

Genus II. *Alysiella* Langeron 1923, 116[AL]

TONE TØNJUM

A.ly.si.el′la. Gr. fem. n. *alysion* small chain; M.L. *-ella* dim. ending; M.L. fem. n. *Alysiella* small chain.

Organisms that exist in characteristic **flat, ribbon-like multicellular filaments**. The **long axis of the individual cells is perpendicular to the long axis of the filament**. The **cells within the filament are paired**, and in axenic culture the filament often breaks up into groups of two or four cells. The width of an individual cell (the width of a filament) is about 2.0–3.0 μm, and the length of a cell is about 0.6 μm. The length of the filament is quite variable. The filament does not show either a dorsal-ventral differentiation or a convex-concave curvature in transverse cross-section. The **ends of the individual filaments are square**. Gram negative. **Motile by gliding** of the entire filament in the direction of the long axis. Aerobic. Chemoorganotrophic. Some may produce acid aerobically from carbohydrates. Optimal temperature: 37°C. Found in the oral cavity of warm-blooded vertebrates

The mol% G + C of the DNA is: 44–48.

Type species: **Alysiella filiformis** (Schmid *in* Simons 1922) Langeron 1923, 118, *Simonsiella filiformis* Schmid *in* Simons 1922, 509.)

FURTHER DESCRIPTIVE INFORMATION

The filaments of *Alysiella* are distinctive, and members of this genus can be recognized by their morphology alone (Fig. BXII.β.64). The filament is flat and ribbon-shaped rather than cylindrical and consists of continuous pairs of cells (Figs. BXII.β.65 and BXII.β.66). The individual cells are oblong, disk-shaped, and several times greater in width than in length. Fibrils are produced from only one side of the filament (Fig. BXII.β.66), and the fibrils appear to be involved in anchoring the filament to epithelial cells of the oral cavity (Kaiser and Starzyk, 1973; McCowan et al., 1979). Cells anchored in this way give rise to a typical palisade arrangement (Fig. BXII.β.64).
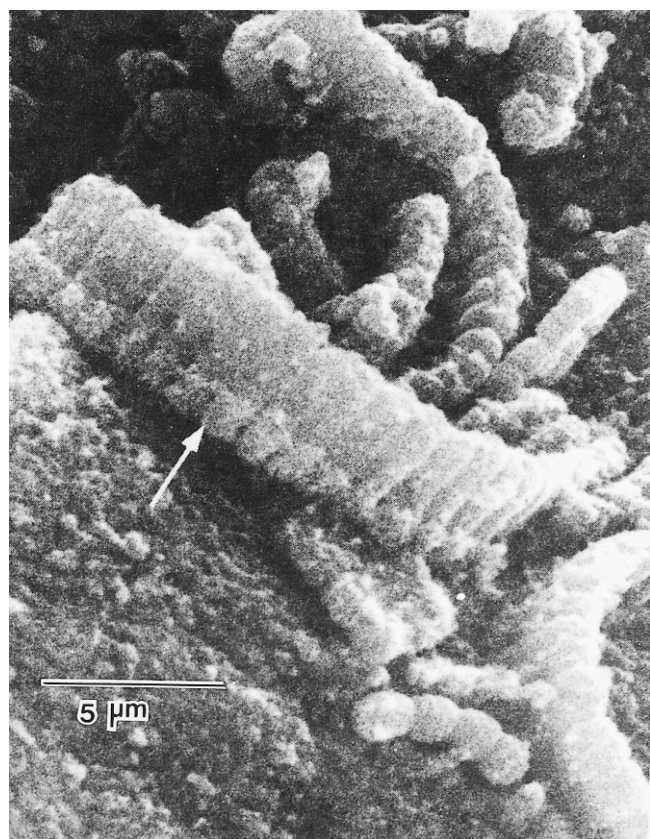
FIGURE BXII.β.64. Scanning electron micrograph of an *Alysiella* filament attached to the epithelium of the bovine tongue. The arrow indicates the fringe of fibers that attach the bacterial filament by its side to the substrate. The palisade organization of the filament is characteristic of *Alysiella*. (Reproduced with permission from R.P. McCowen et al., Applied and Environmental Microbiology *37:* 1224–1229, 1979, ©American Society for Microbiology.)

FIGURE BXII.β.65. Longitudinal electron micrograph of *Alysiella* from the oral cavity of a rabbit. The paired nature of the cells within the filament is typical of the genus. (Reproduced with permission from G.E. Kaiser and M.J. Starzyk, Canadian Journal of Microbiology *19:* 325–327, 1973, ©National Research Council of Canada.)

Gliding motility occurs when the flat surface of the filament is in contact with an agar surface. No organs of locomotion have been detected. Colonies on Oxoid nutrient agar containing 10% horse or ox serum are nonpigmented and about 1.0–1.5 mm in diameter (Steed, 1962). On BSTSY agar (see *Simonsiella* for ingredients), the colonies are about 1.0–2.0 mm in diameter and exhibit a pale yellow pigmentation (Kuhn, 1981). No resting stage has been detected. Generally, the cellular carbohydrate pattern exhibits some similarities with that of *S. muelleri* (Heiske and Mutters, 1994). However, although *A. filiformis* has a cellular carbohydrate pattern different from all other taxa described, the results have to be regarded as preliminary because only the type strain has so far been analyzed.

All reports of *Alysiella* indicate that it is restricted to the oral cavity of warm-blooded vertebrates, where it apparently is nonpathogenic.

ENRICHMENT AND ISOLATION PROCEDURES

At the present time there is no enrichment procedure for *Alysiella*. Direct isolation from the oral cavity can be achieved; however, Steed (1962) isolated *Alysiella* from sheep and rabbits with oral swabs that were plated directly onto Oxoid nutrient agar containing 10% horse or ox serum. After 6 h the microcolonies were transferred to new media using a micromanipulator.

McCowan et al. (1979) isolated *Alysiella* from cows by im-

pressing a portion of tongue on the agar surface and then spreading the organisms or by washing a portion of tongue in phosphate-buffered saline, homogenizing the tongue with a Waring blender, and plating the resulting suspension on agar. They used both Tryptose-blood agar (Difco Laboratories) with 10% sheep blood or an agar consisting of the following ingredients: nutrient agar (Difco), 2.5%; sodium acetate, 0.01%; and yeast extract (Difco), 0.5%.

Kuhn (1981) suggested that *Alysiella* can be isolated on BSTSY agar by using the procedures that she used for isolating *Simonsiella* (see the description of the genus *Simonsiella*).

MAINTENANCE PROCEDURES

Alysiella should be grown at 37°C, and freshly isolated cultures should be transferred at intervals of 2–3 days. Older cultures must be transferred at weekly intervals.

Refrigeration is not recommended for preservation of cultures, but they can be preserved by freezing in liquid nitrogen using glycerol as a cryoprotectant or by lyophilization.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

The procedures that should be used for characterizing isolates of *Alysiella* are identical to those used for *Simonsiella*, and they are presented in the description of that genus.

DIFFERENTIATION OF THE GENUS *ALYSIELLA* FROM OTHER GENERA

The unusual morphology of the *Alysiella* filaments serves to differentiate the genus from all other procaryotic organisms. *Alysiella* filaments differ from *Simonsiella* in consisting of continuous pairs of cells instead of being segmented into units of eight cells,
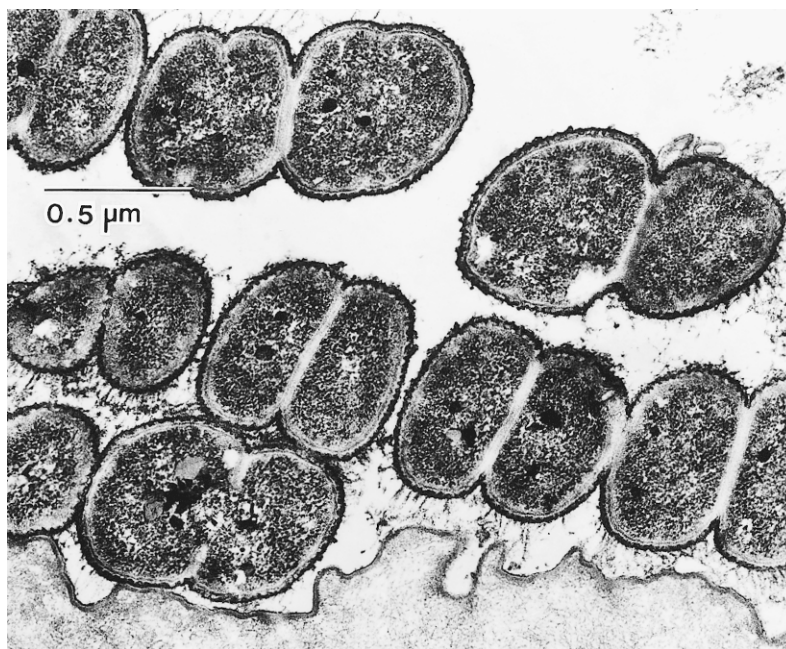
FIGURE BXII.β.66. Section of *Alysiella* from the bovine tongue, showing ruthenium red-stained fibrils emanating from one side of the filament only. (Photo courtesy of J.W. Costerton.)

and the terminal cells are not rounded, but square. In addition, *Alysiella* has a fringe of fibers on the side of the filament rather than on the bottom as in *Simonsiella*. Furthermore, *Alysiella* does not show the dorsal–ventral, convex–concave curvature of *Simonsiella*.

TAXONOMIC COMMENTS

Alysiella has been reported from the oral cavity of many animals including chickens, sheep, horses, cows, goats, pigs, rabbits, and guinea pigs. Isolates have only been obtained from guinea pigs (Berger, 1963), sheep (Steed, 1962), and cows (McCowan et al., 1979); only a few strains from sheep have been described in detail. Strains from other animals have resisted isolation. It is probable that as strains from other animals are isolated, there will prove to be additional species, just as with *Simonsiella*. The

analysis of misnamed *Alysiella* strains has confused the taxonomic work on this entity (Rossau et al., 1989). However, the combined and repeated findings on *Alysiella* morphology (Kaiser and Starzyk, 1973), cellular carbohydrate pattern (Heiske and Mutters, 1994), ribosomal RNA hybridization (Rossau et al., 1989), and 16S rDNA sequence analysis on the verified type strain clearly show that *Alysiella* belongs to the family *Neisseriaceae*. The complete data also indicate that *Alysiella* is a separate genus within this family, closely affiliated with the genus *Simonsiella* (see Fig. BXII.β.76 under chapter on the genus *Simonsiella* and Fig. BXII.β.61 under chapter on the order *Neisseriales*).

ACKNOWLEDGMENTS

The information provided in the first version of *Bergey's Manual of Systematic Bacteriology* (1984) by J.M. Larkin is greatly acknowledged.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *ALYSIELLA*

Only a single species of *Alysiella* is currently recognized. The multicellular spore formers found in the rumen of domestic animals and in the cecum of guinea pigs that Grassè (1924) termed

Alysiella filiformis are probably members of the genus *Arthromitus* (Kuhn, 1981). Some strains in the literature have been misnamed as *A. filiformis* (Rossau et al., 1989).

List of species of the genus Alysiella

1. **Alysiella filiformis** (Schmid *in* Simons 1922) Langeron 1923, 118[AL] *Simonsiella filiformis* Schmid *in* Simons 1922, 509.)

   fi.li.for′ mis. L. n. *filum* thread; L. n. *forma* shape; M.L. adj. *filiformis* filiform.

   See the generic description for additional features. *Alysiella* is aerobic, possesses cytochrome oxidase, and produces catalase. Good growth occurs between 33° and 40°C, with an optimal temperature at 37°C. Growth also occurs at 43°C but not at 27°C. This organism grows in the presence of 1% but not 1.5% NaCl. Grows at pH 7.3 and 9.0 but not 6.0. Acid is produced from D-fructose, α-D-glucose, maltose,

   sucrose, and trehalose. No acid is produced from the following: L-arabinose, cellobiose, dulcitol, erythritol, D-galactose, glycerol, inositol, α-lactose, mannose, melibiose, melizitose, raffinose, rhamnose, salicin, sorbitol, sorbose, or xylose. Variable results occur on inulin and ribose. A rich medium containing 10% serum is best for growth. A slight hydrolysis of gelatin may occur, but agar, casein, starch, esculin, and hippurate are not hydrolyzed. No change occurs in litmus milk. Indole, MR, VP, and reduction of nitrates are negative. Urease is not produced. H_2S production is variable and inconsistent. Hemolytic on rabbit or horse blood agar. The type strain was isolated from sheep saliva.

The mol% G + C of the DNA is: 44–48 (T_m, Bd).

Type strain: ATCC 15532, ATCC 29469, CIP 103342, ICPB 3653, HIM 928-7, NCTC 10282.

GenBank accession number (16S rRNA): AF487710.

Genus III. **Aquaspirillum** Hylemon, Wells, Krieg and Jannasch 1973b, 36[AL]*

BRUNO POT AND MONIQUE GILLIS

Aq.ua.spi.ril' lum. L. *aqua* water; Gr. n. *spira* a spiral; N.L. dim. neut. n. *spirillum* a small spiral; *Aquaspirillum* a small water spiral.

Rigid, helical cells, 0.2–1.4 μm in diameter; however, one species is vibrioid and one species contains straight-to-curved rods. A polar membrane underlies the cytoplasmic membrane at the cell poles in all species so far examined for this characteristic by electron microscopy. **Intracellular poly-β-hydroxybutyrate** is formed, except in two species. Some species form thin-walled coccoid bodies, which predominate in cultures of three to four weeks. **Gram negative. Motile by polar flagella, generally bipolar tufts**; one species is monotrichous, others have a single flagellum at one or at each pole. **Aerobic to microaerophilic**, having a respiratory type of metabolism with **oxygen as the terminal electron acceptor**; a few species can grow anaerobically with nitrate. The optimal growth temperature for most species is 30–32°C. **Chemoorganotrophic**; however, one species is a facultative hydrogen autotroph. **Oxidase positive. Usually catalase and phosphatase positive. Indole and sulfatase negative**. Casein, starch, and hippurate are not hydrolyzed. No growth occurs in the presence of 3% NaCl. A few species can denitrify. Nitrogenase activity occurs in some species, but only under microaerobic conditions. Carbohydrates are not usually metabolized, but a few species can attack a limited variety. Amino acids or the salts of organic acids serve as carbon sources. Vitamins are not usually required. Usually occur in stagnant, freshwater environments.

The mol% G + C of the DNA is: 49–66.

Type species: **Aquaspirillum serpens** (Müller 1786) Hylemon, Wells, Krieg and Jannasch 1973b, 366 (*"Vibrio serpens"* Müller 1786, 48.)

FURTHER DESCRIPTIVE INFORMATION

Cellular morphology Most species of *Aquaspirillum* have helical cells; however, *A. delicatum* is vibrioid (has less than one complete turn or twist). Variants that are nearly straight rods have been obtained from helical species after prolonged transfer (Terasaki, 1972). For helical aquaspirillae, the cells within a given species have a constant type of helix clockwise (right-handed) or counter-clockwise (left-handed) (Terasaki, 1972). The effect of the beta-lactam antibiotic cephalexin on the spiral conformation has been examined by scanning electron microscopy (Konishi and Yoshii, 1986). *A. itersonii* and *A. peregrinum*, which have a left-handed spiral shape, maintained this shape in elongated cells in medium containing cephalexin. The spiral conformation of the elongated cells is therefore considered to represent the natural condition (Konishi and Yoshii, 1986). Photographs showing the comparative size and shape of various aquaspirillae are presented in Fig. BXII.β.67.

Although aquaspirillae are more rigid than spirochetes, they do have a certain degree of flexibility. For example, during rapid swimming the helical cells tend to become straighter. Also, cells embedded in glycerol gelatin can be stretched to three times their original length (Isaac and Ware, 1974).

As unusual elaboration of the plasma membrane, the "polar membrane," occurs in all of the species examined so far (I.J. Beveridge and R.G.E. Murray, unpublished results). It is attached to the inside of the plasma membrane by bar-like links and is most commonly located in the region surrounding the polar flagella (Murray and Birch-Andersen, 1963; Fig. BXII.β.68). Such a membrane has been found mainly in genera of helical bacteria, such as *Spirillum, Oceanospirillum, Campylobacter, Ectothiorhodospira,* and *Rhodospirillum.*

Intracellular poly-β-hydroxybutyrate occurs in all species except *A. gracile* and *A. psychrophilum.* The granules of this polymer stain with metachromatic dyes such as toluidine blue (Martinez, 1963), as well as with lipid-soluble dyes such as Sudan black.

In certain species, the cells develop into thin-walled coccoid bodies (sometimes termed "microcysts") within several days to several weeks. All species may show a few such forms in old cultures, but in *A. itersonii, A. peregrinum* subsp. *peregrinum,* and *A. polymorphum* they predominate. Such coccoid bodies are also formed by members of the genus *Oceanospirillum* and the genus *Campylobacter.* In *A. itersonii,* the development of the helical cells into coccoid bodies can be greatly accelerated by treatment with mitomycin or ultraviolet light; this effect has been correlated with the induction of a defective bacteriophage (Clark-Walker, 1969). Whether the coccoid bodies of aquaspirillae are resistant to desiccation, or whether they are viable, is not known.

Most species of *Aquaspirillum* are motile by means of bipolar tufts or fascicles of flagella; however, *A. delicatum* usually has 1–2 flagella at a single pole, and *A. polymorphum* has bipolar single flagellum. Aquaspirillae generally have flagella that are crescent shaped or that have a long wavelength (over 3 μm), with less than one complete wave. Such flagella are especially likely to occur with the larger aquaspirillae such as *A. serpens, A. metamorphum,* and *A. putridiconchylium,* and the motility of such spirilla is similar to that described for *Spirillum volutans* (i.e., the flagellar fascicles form cones of revolution). However, some aquaspirillae, especially those that are small or medium in cell diameter such as *A. dispar* or *A. delicatum,* have more conventional, helical flagella (see Hylemon et al., 1973b).

A. serpens has been studied extensively with regard to its flagella cell-wall association (Coulton and Murray, 1978), cell-wall ultrastructure, and cell-wall chemical composition (Murray et al.

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*Editorial Note: Phylogenetically, the genus *Aquaspirillum* is heterogeneous; however, it is difficult to definitively delineate new genera for potentially misclassified species. Therefore these organisms are retained in the genus *Aquaspirillum.* The following species are not true aquaspirillae (i.e., not phylogenetically related to the type species, *Aquaspirillum serpens,* at the genus level) but have not been transferred to other genera: *A. anulus, A. autotrophicum, A. delicatum, A. dispar, A. giesbergeri, A. gracile, A. metamorphum, A. polymorphum, A. psychrophilum, A. putridiconchylium,* and *A. sinuosum*. Aquaspirillae that have been generically renamed since the first edition of the *Bergey's Manual of Systematic Bacteriology* (Krieg, 1984a), or for which a new genus name is proposed here, include: *Aquaspirillum aquaticum, A. bengal, A. fasciculus, A. itersonii, A. magnetotacticum,* and *A. peregrinum.*
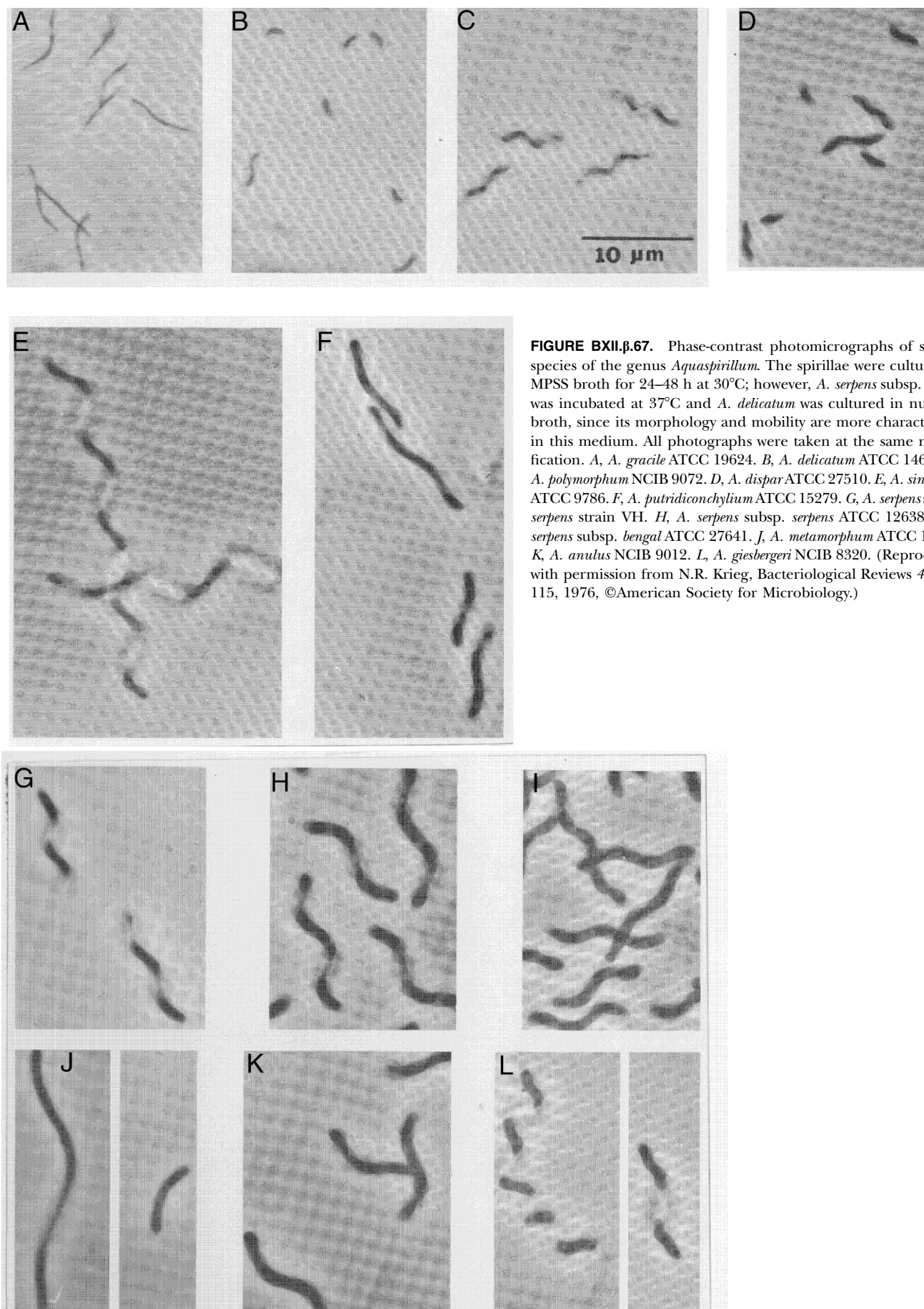
FIGURE BXII.β.67. Phase-contrast photomicrographs of several species of the genus *Aquaspirillum*. The spirillae were cultured in MPSS broth for 24–48 h at 30°C; however, *A. serpens* subsp. *bengal* was incubated at 37°C and *A. delicatum* was cultured in nutrient broth, since its morphology and mobility are more characteristic in this medium. All photographs were taken at the same magnification. *A, A. gracile* ATCC 19624. *B, A. delicatum* ATCC 14667. *C, A. polymorphum* NCIB 9072. *D, A. dispar* ATCC 27510. *E, A. sinuosum* ATCC 9786. *F, A. putridiconchylium* ATCC 15279. *G, A. serpens* subsp. *serpens* strain VH. *H, A. serpens* subsp. *serpens* ATCC 12638. *I, A. serpens* subsp. *bengal* ATCC 27641. *J, A. metamorphum* ATCC 15280. *K, A. anulus* NCIB 9012. *L, A. giesbergeri* NCIB 8320. (Reproduced with permission from N.R. Krieg, Bacteriological Reviews *40:* 55–115, 1976, ©American Society for Microbiology.)
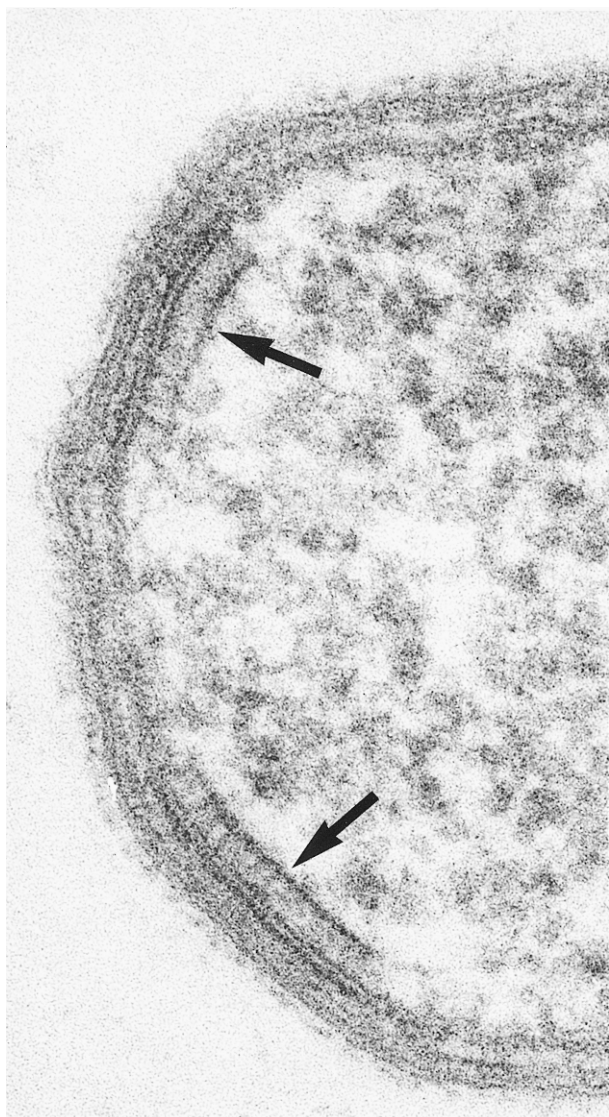
FIGURE BXII.β.68. Thin-section through the polar region of a cell of *Aquaspirillum serpens* strain VHA, showing the polar membrane (*arrows*). A protein layer can also been seen external to the outer wall membrane. × 262,000. (Reproduced with permission from R.G.E. Murray, University of Western Ontario, London, Canada.)

1965; Chester and Murray, 1975, 1978). A protein layer, consisting of a regular array or mosaic of subunits, surrounds the cell walls of certain species of aquaspirillae (Buckmire and Murray, 1970; Beveridge and Murray, 1976; Stewart et al., 1980). Such protein layers can be dissociated by agents such as sodium dodecyl sulfate or guanidine, and can subsequently be reassembled onto templates *in vitro* in the presence of Ca^{2+}. One function of the protein layer of *A. serpens* is to protect against attack by bdellovibrios (Buckmire, 1971). The cell wall lipopolysaccharide of *A. serpens* differs from that of the majority of other Gram-negative bacteria in that it lacks 2-keto-3-deoxyoctonic acid (Chester and Murray, 1975); this compound is also present in the lipopolysaccharide of *A. itersonii* and *A. peregrinum*. In *A. serpens*, lipid A of the lipopolysaccharide differs from that found in members of *Enterobacteriaceae* in that 3-hydroxydodecanoic acid, rather than 3-hydroxytetradecanoic acid, is the *N*-acylating acid.

Physiology All species of *Aquaspirillum* are aerobic. Although *A. itersonii* and *A. peregrinum* can acidify fructose-containing media sealed with a layer of oil or petrolatum, significant turbid growth does not occur, and these species should be considered to have an essentially oxidative type of metabolism. *A. itersonii*, *A. dispar*, and *A. psychrophilum* can grow anaerobically using nitrate and possess a dissimilatory nitrate reductase. *A. itersonii*, *A. dispar*, and *A. psychrophilum* can reduce nitrate beyond nitrite, but only *A. psychrophilum* appears to form visible amounts of gas from nitrate (Terasaki, 1972, 1979).

The respiratory chain of *A. itersonii* has been studied in detail and appears to be an unbranched, membrane-bound electron transport chain from NADH and succinate to oxygen (Dailey, 1976). Cytochromes of both the *b* and *c* type, but not of the *a* type, are present, and their biosynthesis and properties have been investigated (Clark-Walker et al., 1967; Clark-Walker and Lascelles, 1970; Ho and Lascelles, 1971; Dailey and Lascelles, 1974). *A. itersonii* synthesizes higher levels of cytochromes *b* and *c* under semi-anaerobic conditions with nitrate in the medium than it does anaerobically without nitrate (Clark-Walker et al., 1967), and much of the cytochrome *c* is present in a soluble form in the periplasmic space (Gauthier et al., 1970). This soluble cytochrome *c*, and other periplasmic proteins, can be selectively liberated from the cells by the use of a mixture of Tris buffer and EDTA (Garrard, 1971). Biosynthesis of the soluble cytochrome *c* has been investigated by Garrard (1972). The complete amino acid sequence of the cytochrome c_{550} from *A. itersonii* has been elucidated (Woolley, 1987). The sequence is a single polypeptide chain of 111 residues and shows a high degree of sequence homology with the cytochrome c_2 from the photosynthetic bacterium *Rhodospirillum rubrum*. This homology is in agreement with other phylogenetic data, as shown below.

Aquaspirillum species cannot grow in the presence of 3% NaCl, and many species cannot tolerate even 1% NaCl. This lack of salt tolerance distinguishes *Aquaspirillum* from *Oceanospirillum*, because the latter genus requires seawater or Na^+ for growth.

Commonly used culture media for aquaspirillae are PSS broth, MPSS broth, and nutrient broth[1]. Aquaspirillae generally produce moderate-to-abundant turbid growth in 2–3 d in PSS broth (Hylemon et al., 1973b). In nutrient broth, membranous masses are formed at the surface and can be dispersed with shaking to yield turbid cultures (Terasaki, 1972).

Colonies of aquaspirillae generally develop within 2–3 d on PSS agar and are usually white, circular, and convex, ranging from pinpoint to 1.5 mm in diameter (Hylemon et al., 1973b). Colonies on nutrient agar are generally pinpoint in size at 48 h but become larger (up to 2.0 mm in diameter) at 7 d; they are usually convex or umbonate, glistening, opaque, pale yellow, and butyrous (Terasaki, 1972). S–R variation has been found in several species (Terasaki, 1972). Some species produce a water-soluble, yellow-green, fluorescent pigment on PSS agar.

Most species grow best at 30–32°C, except *A. psychrophilum*, which grows best at 20°C and cannot grow above 26°C, and *A. bengal*, which grows best at 41°C. The optimal pH for most species

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1. PSS broth (g/l): Bacto Peptone (Difco), 10.0; succinic acid (free acid), 1.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4·7H_2O$, 1.0; $FeCl_3·6H_2O$, 0.002; and $MnSO_4·H_2O$, 0.002. The pH is adjusted to 6.8 with KOH before autoclaving the medium. For PSS agar, 16.0 g of agar is added/l; for PSS semisolid medium, 1.5 g of agar is added/l. For MPSS media, use 5.0 g of peptone rather than 10.0 g. Nutrient broth (g/l): peptone, 5.0; meat extract, 3.0 g; the pH is adjusted to 7.0–7.2 with KOH before autoclaving. For nutrient agar, 15.0 g/l of agar is added.

is 6.5–7.5, but many species can grow at pH values as high as 8.5 or 9.0 (Terasaki, 1972).

The nutrition of aquaspirillae is generally simple. Most species grow in simple defined media with amino acids or the salts of organic acids as carbon sources and ammonium salts as the nitrogen source. Only *A. gracile* is known to require biotin. Few species can catabolize sugars, although a limited set of sugars can be used by *A. gracile*, *A. itersonii*, and *A. peregrinum*. Acidification of sugar media by these species occurs only when the peptone level is kept low (0.2% or less). A listing of the carbon sources for aquaspirillae is given in Table BXII.β.79. In this table, some contradictions exist between the results obtained in different laboratories, although the intralaboratory results are reproducible. These contradictions are likely due to the differences in methodology, to the definition of what constitutes a positive growth response, and, in some cases, to the use of different strains.

It is clear that the genus *Aquaspirillum* is quite heterogeneous (see below). Some of the phenotypic and nutritional differences confirm the genotypic differentiation. The subdivision of the species in the Tables BXII.β.79, BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83 corresponds with the genotypic groupings obtained from 16S rRNA sequencing and rRNA–DNA hybridization data, as discussed below.

A. peregrinum exhibits nitrogenase activity, but only under microaerobic conditions (Strength et al., 1976). In this respect, it is similar to members of the genus *Azospirillum*. Atmospheric nitrogen fixation has also been reported in certain strains of *Aquaspirillum itersonii* (Ketkar, 1967; Ketkar and Dhala, 1978). Phylogenetic data also suggest that both species are phylogenetically more closely related to members of the genus *Azospirillum* than to other species of the genus *Aquaspirillum*.

The intermediary metabolism of sugars has been studied in *A. itersonii* and *A. gracile*. *A. itersonii* can acidify glucose media under semi-anaerobic conditions, but not under aerobic conditions (Terasaki, 1972, 1979); this observation has not yet been explained. Under aerobic conditions, *A. itersonii* is impermeable to glucose despite the occurrence of high levels of glucokinase activity (Hylemon et al., 1974). Fructose is transported and phosphorylated by means of a fructose-specific phosphoenolpyruvate phosphotransferase system (P.V. Phibbs, unpublished results). The Embden–Meyerhof–Parnas and Entner–Doudoroff pathways occur in *A. itersonii* and *A. gracile*, but the hexose monophosphate pathway is absent (Hylemon et al., 1974; Laughon and Krieg, 1974). *A. gracile* acidifies sugar media by formation of sugar acids, such as gluconic acid; other organic acids are not formed (Laughon and Krieg, 1974).

The tricarboxylic acid cycle has been demonstrated in *A. serpens* and *A. itersonii* (Cole and Rittenberg, 1971). Whether the glyoxylate shunt occurs is not known.

Serology McElroy and Krieg (1972) reported serological differentiation of most species of *Aquaspirillum*. Antisera are prepared against whole cells and adsorbed with heated cells, leaving only antibodies against thermolabile cell components. The use of such antisera in agglutination tests with a limited number of strains suggests that most species can be distinguished from one another and from organisms of other genera.

Genetics Electroporation methods and conjugal mating have been used to transfer several plasmid vectors (including the incompatibility P class plasmid RP4 by conjugation from *Escherichia coli* HB101) to *A. dispar* and *A. itersonii*. The transconjugants

were able to donate the plasmid to plasmid-free *E. coli* and *A. dispar* strains by conjugal mating (Eden and Blakemore, 1991). *A. dispar* and *A. itersonii* were transformed at efficiencies as high as 3×10^4 transformants/µg plasmid DNA by high-voltage electrotransformation. RP4 DNA from *Spirillum* hosts, but not RP4 from *E. coli*, was successfully transferred to *A. dispar* and to *A. itersonii*, indicating the possible presence of a restriction/modification system in these *Aquaspirillum* species. The restriction endonuclease *Ase*I has been isolated from *A. serpens*. This enzyme recognizes the sequence 5′-AT–TAAT-3′ (Polisson and Morgan, 1988).

There has been only one report of bacteriophages for *Aquaspirillum*. An icosahedral, double-stranded DNA phage specific for a particular strain of *A. itersonii* was isolated from raw sewage in Australia by Clark-Walker and Primrose (1971). It produced plaques on plate cultures, but was unable to lyse broth cultures. Oddly, the host strain of *A. itersonii* was originally isolated from Lake Erie, U.S.A., rather than from Australia.

Ecology Aquaspirillae are considered to be nonpathogenic for humans and animals. An organism known as *"Spirillum minus"* (see *Species Incertae Sedis*) is the cause of one of the two forms of rat-bite fever in man, and another organism, *"Spirillum pulli"* (see *Species Incertae Sedis*), is apparently the cause of a diphtheritic stomatitis in chickens. Neither of these species belong to the genus *Spirillum* or to the genus *Aquaspirillum*, and their affiliation with other established genera is uncertain. A few cases of human infection have been reported to be caused by, or associated with, organisms resembling aquaspirillae (e.g., see Edwards and Kraus, 1960; Kowal, 1961). The identity of these strains is uncertain.

A total of seven strains were isolated from blood cultures of six patients and from the cerebrospinal fluid of one patient (five cases of pneumonia and two cases of pneumonia associated with meningitis). These unusual Gram-negative rods had highest protein profile similarity with *A. serpens* and *Chromobacterium violaceum* (Casalta et al., 1989), as measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). However, no serologic cross-reactions were observed (Western blot and immunofluorescence), and considerable differences in morphological and biochemical characteristics were seen. It was concluded that additional studies, including DNA–DNA hybridization, were required to classify this bacterium.

Spirillum-like organisms have also been detected in diseased mosquito larvae (Fulton et al., 1974), but whether they are aquaspirillae is uncertain. Giant spore-forming spirillae have been described in the intestinal contents of tadpoles by Delaporte (1964) (see *Genus Incertae Sedis "Sporospirillum"*); little is known of these organisms, but they do not appear to be *Aquaspirillum*. Tiunov et al. (1997) also reported that bacteria of the genera *Aquaspirillum* and *Cytophaga* invariably predominated in the saprotrophic bacterial community at 2 mm distance from the burrow wall of the earthworm *Lumbricus terrestris* (drilosphere). The earthworm burrow walls differed from the surrounding soil by the high density of bacteria (10 times more, or $15–20 \times 10^6$ CFU/g, at 7–10 cm depth) and by the composition. Spirillae predominated in the 2 mm zone around the burrow walls over the entire experimentation period. At distances of 5 and 10 mm from the burrow, the taxonomic structure of the bacterial community was highly diverse and varied significantly depending on the season. Identification, however, was based only on microscopic examination of isolated colonies; therefore possible con-

TABLE BXII.β.79. Carbon sources used by existing and former species of *Aquaspirillum*

	Aquaspirillum spp. from the *Betaproteobacteria*													
	Aquaspirillum (sensu stricto)			*Aquaspirillum* spp. (phylogenetically not correctly named)				*Aquaspirillum* spp. belonging to the *Comamonadaceae*						
	A. serpens subsp. *serpens*		*A. serpens* subsp. *bengal*[c]	*A. autotrophicum*[d]	*A. dispar*[a]	*A. putridiconchylium*		*A. anulus*		*A. delicatum*		*A. gracile*[e]	*A. giesbergeri*	
Carbon source	A[a]	B[b]				A[a]	B[b]	A[a]	B[b]	A[a]	B[b]		A[a]	B[b]
Citrate	−	−	−	+	+	−		−	−	−	−	−	−	−
Aconitate	−			+	+	+				+			−	
Isocitrate	−			+	+	+				+			−	
α-Ketoglutarate	d			+	+	+				+		+	−	
Succinate	−	+	+	+	+	+	d	−	w	+	w	+	−	d
Fumarate	−	+	+	+	+	+	+	−	w	+	w	−	d	d
Malate	−	d	+	+	+	+	+	−	d	+	w	−	−	d
Oxaloacetate	d			+	+	+		−		+		−		
Pyruvate	d	+	+	+	+	+	+	d	d	+	w	+	+	d
Lactate	−	d	+	+	+	+	d	−	w	+	w	+	−	d
Malonate	−	−		+	+	+	−	w	−	+	w	−		d
Tartrate		−		+			−	−		−				−
Acetate	−	d	+	+	+	+	w	−	w	+	w	+	−	d
Propionate	−	d		+	+	−	d	−	−	−	−	−	−	−
Butyrate		+		+			+		d		w			d
Caproate	−			−	+	−		−		−		−		
β-Hydroxybutyrate	−			+	+	+		−		−		−		
p-Hydroxybenzoate	−			+	−	−		−		−		−		
Ethanol	−	−	−	−	d	−	w	−	−	−	w	−	−	−
n-Propanol	−	−	−	−	−	−	w	−	−	−	−	−	−	−
n-Butanol	−	−	−	−	−	−	w	−	−	−	w	−	−	−
Glycerol	−	−	−	−	−	−	−	−	−	−	w	+	−	−
D-Fructose	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Glucose	−	−	−	−	−	−	−	−	−	−	−	+	−	−
D-Xylose	−	−	−	−	−	−	−	−	−	−	−	+	−	−
L-Arabinose	−	−	−	−	−	−	−	−	−	−	−	+	−	−
L-Histidine	−				+			−		−		−		
L-Tyrosine	−			+	−			−		−		−		
L-Phenylalanine	−			+				−		−		−		
L-Alanine	d	+		+	+	+		−		−		−		
L-Glutamate	+	+		+	d	+		+		−		+	−	
L-Aspartate	+	+		+	+	+		−		−		+	−	
L-Glutamine	d	+		+	−	+		−		−		−		
Asparagine	d	+		+	−	+		−		−		−		
L-Proline	d	+		+	+	+		−		−		−		
L-Hydroxyproline	−				−	−						−		
L-Ornithine	−			−	+			−		−		−		
L-Citrulline	−			−	+			−		−		−		
L-Arginine	−		−	−	−			−		−		−		
L-Lysine	−		−	−	−			−		−		−		
Putrescine	−				−	−		−		+		−		
L-Methionine	−		−	−	−			−		−		−		
L-Serine	d		−	−	−			−		−		−		
L-Cysteine	−		−	−	−			−		−		−		
Glycine	−		−	+	−			−		−		−		
L-Leucine	−		−	−	−			−		−		−		
L-Isoleucine	−		−	+	−			−		−		−		
L-Valine	−		−	−	−			−		−		−		
L-Tryptophan	−			+	−			−		−		−		

[a]As determined by the method of Hylemon et al. (1973b). A turbidimetrically standardized cell suspension in physiological saline was inoculated into a defined, vitamin-free medium containing the carbon sources (0.1%) and ammonium sulfate as the nitrogen source. Growth responses were measured turbidimetrically after one 72-h serial transfer from the initial cultures, using a Klett colorimeter with the blue (420 nm) filter and 16-mm cuvettes. Symbols: +, 10 or more Klett units of turbidity for all strains tested; −, less than 10 Klett units of turbidity; d, differs among strains; blank space, not determined.

[b]As determined by the method of Terasaki (1972, 1979). A cell suspension washed in basal, defined, vitamin-free medium (Williams and Rittenberg, 1957) lacking carbon sources was inoculated into similar media containing the test compounds (0.05%) and ammonium chloride as the nitrogen source. After 7 days, growth was estimated turbidimetrically. Symbols: +, a turbidity of 0.025 absorbance units or greater for all strains tested; w, a turbidity of less than 0.025; −, no growth (turbidity equals the same as the appearance of controls without a carbon source); d, differs among strains; blank space, not determined.

[c]As determined by Kumar et al. (1974), using a modification of the method of Hylemon et al. (1973b). Symbols: +, a turbidity of 0.03 absorbance units or more, using a green filter and 16-mm cuvettes; −, turbidity less than 0.03; blank space, not determined.

[d]The utilization of compounds as sole carbon sources was tested on agar plates as described by Stanier et al. (1966) using a velvet-disk replicator. The medium was the basal mineral agar described under Procedures for Testing Special Characters, supplemented with 0.2% carbohydrates or 0.1% of other compounds. Symbols: +. growth greater than on control plate with no carbon source; −, growth no greater than on control plate; blank space, not determined.

[e]As determined by the method of Canale-Parola et al. (1966). A complex growth-limiting medium containing the carbon sources at 0.5% was used. Growth in the presence of the test compounds was compared turbidimetrically or by microscopic count to that occurring in the absence of the compounds. Symbols: +, >10% increase in the growth of all strains in the presence of the test compound; −, 10% or less increase in growth; blank space, not determined.

(continued)

TABLE BXII.β.79. *(cont.)*

Carbon source	*Aquaspirillum* spp. belonging to the *Comamonadaceae* — *A. metamorphum* A[a]	B[b]	*A. psychrophilum*[b,f]	*A. sinuosum* A[a]	B[b]	*A. polymorphum* A[a]	B[b]	*Levispirillum itersonii* A[a,g]	B[b]	*Levispirillum peregrinum* A[a,h]	B[b]	*Comamonas terrigena* biovar *aquaticum*[i]	*Prolinoborus fasciculus*[j]
Citrate	−	w	−	−	−	−	−		d	−	+	−	−
Aconitate	−			−		−		+		+			
Isocitrate	−			−		−		−		−			
α-Ketoglutarate	+			−		−		+		+		+	−
Succinate	+	w	−	−	w	−	+	+	d	+	+	+	+
Fumarate	+	d	−	−	w	−	+	+	d	+	+	+	+
Malate	+	w	−	+	w	−	+	+	d	+	+	+	+
Oxaloacetate	+			+		−			d	+			+
Pyruvate	+	w	−	+	w	−	+	d	d	+	+	+	+
Lactate	+	w	−	−	w	−	+	d	d	+	+	+	+
Malonate	+	−	−	−	−	+	w	d	−	+	+	−	−
Tartrate		−	−		−		+		−		−	−	−
Acetate	−	w	−	−	w	+	w	−	+	+	+	+	−
Propionate	−	d	−	−	−	−	w	d	d	−	+	+	−
Butyrate		w	−		d	−			d		+		
Caproate	−			−		−		−		−			
β-Hydroxybutyrate	−			−		−		+		+		+	+
p-Hydroxybenzoate	−			−				−		−			
Ethanol	−	−	−	−	−	−	−	+	d	−	+	−	−
n-Propanol	−	−	−	−	−	−	−	+	d	−	+	−	−
n-Butanol	−	−	−	−	−	−	−	d	d	−	+	−	−
Glycerol	−	−	−	−	−	−	−	d	+	−	d	−	−
D-Fructose	−	−	−	−	−	−	−	+	+	+	+	−	−
D-Glucose	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Xylose	−	−	−	−	−	−	−	−	−	−	−	−	−
L-Arabinose	−	−	−	−	−	−	−	−	−	−	−		
L-Histidine	−			−		−		+		−		−	−
L-Tyrosine	−			−		−		−		−		−	−
L-Phenylalanine	−			−		−		+		−		−	−
L-Alanine	+			−		−		d		+		−	+
L-Glutamate	+			−		+		+		+		+	+
L-Aspartate	+			−		+		+		+		+	+
L-Glutamine	+			−		+		+		+		+	+
Asparagine	+			−		−		+		+		+	+
L-Proline	−			−		+		+		+		+	+
L-Hydroxyproline	−			−		−		d		+		−	−
L-Ornithine	−			−		−		d		−		−	−
L-Citrulline	−			−		−		d		−			
L-Arginine	−			−		−		d		−		−	+
L-Lysine	−			−		−		d		−			
Putrescine	−			−		−		d		−			
L-Methionine	−			−		−		−		−			−
L-Serine	−			−		−		−		−			−
L-Cysteine	−			−		−		d		−		−	−
Glycine	−			−		−		−		−			
L-Leucine	−			−		−		d		−			
L-Isoleucine	−			−		−		−		−			
L-Valine	−			−		−		d		−			
L-Tryptophan	−			−		−		−		−			

[f]The nutritional requirements of *A. psychrophilum* have not yet been determined.

[g]Strains of subsp. *nipponicum* were not tested.

[h]Strains of subsp. *integrum* were not tested.

[i]As determined by the method of Kropinski (1975). Samples (0.1 ml) of a washed cell suspension were spread on plates of a minimal agar medium containing ammonium sulfate and niacin. Approximately 8 mg of the test compounds were placed in small areas on the plates. After incubation for 48 h, the growth response was estimated. Symbols: + growth in the area around the test compound; −, no growth; blank space, not determined.

[j]As determined by the method of Strength et al. (1976). A washed, turbidimetrically standardized suspension was inoculated into a defined, vitamin-free, semisolid medium containing the test compounds (on an equal carbon basis relative to 0.2% fumaric acid) and ammonium sulfate as the nitrogen source. Growth responses were measured turbidimetrically at 36 h after gently inverting the semisolid cultures several times to obtain an even distribution of cells. A Klett colorimeter was used (blue filter, 420 nm) with 16 mm cuvettes. Symbols: +, production of at least 10 Klett units of turbidity; −, less than 10 Klett units; blank space, not determined.

TABLE BXII.β.80. Cellular quinone systems in *Aquaspirillum*, *Magnetospirillum*, and former *Aquaspirillum* species[a,b]

Species	Strain number	Group number	Quinone system				
			Q-6	Q-7	Q-8	Q-9	Q-10
Aquaspirillum species from the *Betaproteobacteria*							
Aquaspirillum (*sensu stricto*)							
Aquaspirillum serpens subsp. *serpens*	14924[T]	IV	2	2	96		
Aquaspirillum serpens subsp. *serpens*	14923	IV	16	11	73		
Aquaspirillum serpens subsp. *serpens*	15465	IV	3	2	95		
Aquaspirillum spp. (phylogenetically not correctly named)							
Aquaspirillum autotrophicum	15327[T]	Vf		3	97		
Aquaspirillum dispar	15328[T]	Ve		4	94	2	
Aquaspirillum putridiconchylium	13962[T]	IV	2	2	96		
Aquaspirillum spp. belonging to the *Comamonadaceae*							
Aquaspirillum anulus	14917[T]	Va			98	2	
Aquaspirillum delicatum	14919[T]	Vd	1	1	98		
Aquaspirillum giesbergeri	14959[T]	Va	2	1	96	1	
Aquaspirillum gracile	14920[T]	Vc		1	98	1	
Aquaspirillum metamorphum	13960[T]	Va	1	1	97	1	
Aquaspirillum psychrophilum	13611[T]	Vb	3	1	95	2	
Aquaspirillum sinuosum	14925[T]	Va		1	97	1	1
Aquaspirillum spp. from the *Alphaproteobacteria*							
Aquaspirillum polymorphum	13961[T]	VII			2	3	95
Former *Aquaspirillum* spp.							
Comamonas terrigena biovar aquaticum	14918[T]	Va	1	3	85	11	
Levispirillum peregrinum subsp. *peregrinum*	14922[T]	VI		3	3	93	2
Levispirillum peregrinum subsp. *integrum*	13617[T]	VI		2	4	92	2
Levispirillum itersonii subsp. *itersonii*	14921	VII			1	3	96
Levispirillum itersonii subsp. *nipponicum*[c]	13615[T]	VII			1	6	93
Magnetospirillum							
Magnetospirillum gryphiswaldense	15271[T]	VII			10	4	86
Magnetospirillum magnetotacticum	15272[T]	VII				3	97

[a]Data from Sakane and Yokota (1994).

[b]Numbers refer to the percentage of a quinone system relative to total cellular quinone systems.

[c]Strain ATCC 33333[T] has been shown to be different from strain IFO 13615[T] (Pot, 1996).

fusion with azospirillae cannot be excluded. These "spirillae" are common in soil communities. A similar remark can probably be made regarding the description of some nitrogen-fixing *Aquaspirillum* spp. from the endorhizosphere of rice (Garcia et al., 1983; Mishustin, et al., 1984), sorghum, winter rye, annual rye grass, meadow fescue, timothy grass, meadow soft grass, hogweed cow parsnip, and common colewort growing on different soils (Berestetsky et al., 1985). Genotypic identification is needed to confirm the identity of the *Aquaspirillum* species reported.

Aquaspirillae have been isolated from a wide variety of freshwater sources, especially stagnant ones or those containing organic matter: ditch water, canal water, stagnant ponds, primary oxidation ponds, and eutrophic lakes. They have also been isolated from storage tanks of distilled water in laboratories, where the organisms and the nutrients to support their growth or survival apparently come from the surrounding air. Aquaspirillae have also been isolated from hay infusions made with pond water (the water is probably the source of the organisms) and from putrid infusions of freshwater mussels (where the mud adherent to the shellfish is probably the source).

Aquaspirillae, including strains of *A. itersonii*, have been isolated from soils polluted with chloroanilines (Surovtseva et al., 1996). Under aerobic conditions, these strains were able to grow and degrade 3- and 4-chloroaniline and 3,4-dichloroaniline as sole sources of carbon and nitrogen. Enzymes with different substrate specificity were synthesized during cultivation on different substrates, indicating a potential use for these organisms as bioremediators in soil polluted with chloroanilines (Vasilyeva et al., 1996). Identification of these microorganisms, however, has so far been based only on morphological and physiological characteristics.

Although widely distributed in nature, aquaspirillae comprise only a very small proportion of the total flora of natural habitats. Helical bacteria in general represent only 0.1–0.6% of the flora of pond mud, surface water, slime on stones, or trickling filter effluents, and less than 0.01% in most other habitats (Scully and Dondero, 1973). Consequently, an enrichment procedure is usually necessary before spirillae can be isolated from these habitats.

Chemotaxonomic characteristics   In a comparison of the chemotaxonomic characteristics of 34 spirillae, Sakane and Yokota (1994) found two groups with different nonpolar fatty acid profiles. The first group consisted of aquaspirillae (and oceanospirillae) with the Q-8 quinone system and containing hexadecanoic acid ($C_{16:0}$) and hexadecenoic acid ($C_{16:1}$) as major fatty acids. This group consisted of all the aquaspirillae and oceanospirillae that belonged to the *Betaproteobacteria* and *Gammaproteobacteria*, respectively. A second group, consisting of aquaspirillae, oceanospirillae, and some magnetospirillae, had Q-9 and Q-10 quinones and contained octadecenoic acid ($C_{18:1}$) as a major fatty acid. Phylogenetically all these spirillae belong to the *Alphaproteobacteria*. With regard to the 3-hydroxy fatty acids, more than eight different profiles were found among the 34 spirillae in-

TABLE BXII.β.81. Cellular concentrations of nonpolar and hydroxy fatty acids in *Aquaspirillum*, *Magnetospirillum*, and former *Aquaspirillum* species[a]

Species	Strain[d]	Nonpolar fatty acid[b]													3-hydroxy fatty acids[c]							2-hydroxy fatty acid[e]
		$C_{10:1}$	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{19:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{18:0}$	
Aquaspirillum spp. from the *Betaproteobacteria*																						
Aquaspirillum (sensu stricto)																						
Aquaspirillum serpens subsp. *serpens*	14924[T]		3			1		8	51			3	33				100					−
Aquaspirillum serpens subsp. *serpens*	14923		5			1		9	45	2		7	29				100					−
Aquaspirillum serpens subsp. *serpens*	15465		3		1	2		13	42		1	1	37				100					−
Aquaspirillum spp. (phylogenetically not correctly named)																						
Aquaspirillum autotrophicum	15327T		3					33	33	3	3	1	19	6		19	81					+ ($C_{11:0}$, $C_{18:1}$)
Aquaspirillum dispar	15328T		5		2		4	29	63		3		13				100					−
Aquaspirillum putridiconchylium	13962[T]		1		1			11	73			2	9				100					−
Aquaspirillum spp. belonging to the *Comamonadaceae*																						
Aquaspirillum anulus	14917[T]		2		3		2	33	52			3	4			100						−
Aquaspirillum delicatum	14919[T]		1					32	35	2		1	23	8	100							−
Aquaspirillum giesbergeri	14959[T]		4		4			24	60			1	8			100						−
Aquaspirillum gracile	14920[T]	5						35	50	3		9				58	42					−
Aquaspirillum metamorphum	13960[T]		2		3			25	55	1		1	13			100						−
Aquaspirillum psychrophilum	13611[T]		2		3			19	63	1		2	10		10	90						−
Aquaspirillum sinuosum	14925[T]		2		3			31	58				6			100						−
Aquaspirillum spp. from the *Alphaproteobacteria*																						
Aquaspirillum polymorphum	13961[T]			2	4			20	13			1	58					66		6	28	+ ($C_{18:1}$, $C_{19:0\ iso}$)
Former *Aquaspirillum* spp.																						
Comamonas terrigena biovar aquaticum	14918[T]		2		4		7	28	40	3			16			100						−
Levispirillum pergrinum subsp. *peregrinum*	14922[T]		3					16	19	1	2	2	57					39		45	15	+ ($C_{18:1}$)
Levispirillum pergrinum subsp. *integrum*	13617[T]		2					12	12			2	73					42		34	21	+ ($C_{18:1}$)
Levispirillum itersonii subsp. *itersonii*	14921		4			1		15	13			2	63					40		30	30	+ ($C_{18:1}$)
Levispirillum itersonii subsp. *nipponicum*[f]	13615[T]		3			1		14	20			2	59					42		31	27	+ ($C_{18:1}$)
Magnetospirillum																						
Magnetospirillum gryphiswaldense	15271[T]				3	2		7	25		1	5	56					66		18	16	+ ($C_{18:1}$, $C_{19:0\ iso}$)
Magnetospirillum magnetotacticum	15272[T]		2		1			25	30			1	41					58		39	3	+ ($C_{19:0\ iso}$)

[a] Data from Sakane and Yokota (1994).

[b] The numbers refer to the percentage of an acid relative to the total nonpolar acids.

[c] The numbers refer to the percentage of an acid relative to the total 3-hydroxy acids.

[d] All strain numbers are from the IFO (NBRC) culture collection.

[e] −, absent; +, present

[f] Strain ATCC 33333[T] has been shown to be different from strain IFO 13615[T] (Pot, 1996).

TABLE BXII.β.82. Cellular concentrations of polyamines in *Aquaspirillum*, former *Aquaspirillum* species, *Magnetospirillum*, and *Spirillum*[a,b]

Organism	Strain number (IFO)	Medium	Dap	H-Put	Put	Cad	Spd	HSpd
Aquaspirillum species from the *Betaproteobacteria*								
Aquaspirillum (*sensu stricto*)								
Aquaspirillum serpens subsp. *serpens*	IFO 14923 (ATCC 11335)	199	−	2.00	1.12	−	−	−
		PY	−	0.46	0.94	−	−	−
	IFO 14924[T] (ATCC 12638[T])	PY	−	1.25	1.55	−	−	−
Aquaspirillum serpens subsp. *bengal*	IFO 15485[T] (ATCC 27641[T])	199	−	0.55	0.80	−	−	−
Aquaspirillum spp. (phylogenetically not correctly named)								
Aquaspirillum autotrophicum	IFO 15327[T] (ATCC 29984[T])	199	−	0.65	1.45	−	−	−
Aquaspirillum dispar	IFO 15328[T] (ATCC 27510[T])	199	0.20	0.95	0.01	1.30	−	−
		199[c]	0.09	0.35	0.02	0.51	−	−
Aquaspirillum putridiconchylium	IFO 13962[T] (ATCC 15279[T])	199	−	0.41	0.06	−	−	−
		PY	−	0.40	1.06	−	−	−
Aquaspirillum spp. belonging to the *Comamonadaceae*								
Aquaspirillum anulus	IFO 14917[T] (ATCC 11879[T])	PY	−	1.15	2.00	−	−	−
Aquaspirillum delicatum	IFO 14919[T] (ATCC 14667[T])	PY	−	0.02	0.58	−	−	−
Aquaspirillum giesbergeri	IFO 13959[T] (ATCC 11334[T])	199	−	0.50	0.70	−	−	−
		199[c]	−	0.29	0.76	−	−	−
		PY	−	0.25	0.50	−	−	−
Aquaspirillum gracile	IFO 14920[T] (ATCC 19624[T])	PY	−	1.40	1.10	−	−	−
Aquaspirillum metamorphum	IFO 12012	199	−	1.25	3.00	−	−	−
	IFO 13960[T] (ATCC 15280[T])	199	−	0.77	0.49	−	−	−
Aquaspirillum psychrophilum	IFO 13611[T]	199	−	0.15	0.93	−	0.15	−
		PY	−	0.25	1.54	−	0.08	−
Aquaspirillum sinuosum	IFO 14925[T] (ATCC 9786[T])	PY	−	0.10	0.64	−	−	−
Aquaspirillum spp. from the *Alphaproteobacteria*								
Aquaspirillum polymorphum	IFO 13961[T] (ATCC 11332[T])	199	−	0.30	0.32	−	0.88	0.14
		PY	−	1.75	0.95		0.74	0.50
Former *Aquaspirillum* spp.								
Comamonas terrigena biovar aquaticum	IFO 14918[T] (ATCC 11330[T])	199	−	0.81	1.14	−	0.06	−
		199[c]	−	0.08	0.62	−	0.02	−
Levispirillum peregrinum subsp. *peregrinum*	IFO 14922[T] (ATCC 11332[T])	199	−	−	0.47	−	0.91	−
Levispirillum peregrinum subsp. *integrum*	IFO 13617[T]	199	−	−	0.85	−	0.65	−
		PY	−	−	1.50	−	0.25	−
Levispirillum itersonii subsp. *itersonii*	IFO 14921[T] (ATCC 11331[T])	199	−	−	0.24	−	1.40	0.34
		199[c]	−	−	0.21	−	0.48	0.11
Levispirillum itersonii subsp. *nipponicum*[d]	IFO 13615[T]	199	−	−	0.41	−	0.64	0.26
		199[c]	−	−	1.25	−	0.42	0.14
Magnetospirillum								
Magnetospirillum magnetotacticum	IFO 15272[T] (DSM 3856[T])	ATY	−	−	0.71	0.21	0.21	−
Magnetospirillum gryphiswaldense	IFO 15271[T] (DSM 6361[T])	ATY	−	−	1.30	−	0.33	−
Spirillum								
Spirillum volutans	ATCC 19554[T]	199	−	−	0.20	0.43	0.60	−

[a]Data from Hamana et al. (1994).

[b]Abbreviations: Dap, diaminopropane; H-Put, 2-hydroxyputrescine; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; IFO, Institute for Fermentation, Osaka, Japan; IAM, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; DSM, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; T, type strain; −, not detectable (<0.005).; ATY, growth medium containing 0.1% sodium acetate, 0.05% sodium thioglycolate, 0.01% yeast extract, 0.01% NH_4Cl, 0.01% $MgSO_4·7H_2O$, 0.05% K_2HPO_4, 20 μM ferric citrate, pH 6.9; PY, growth medium containing 1% peptone, 0.2% yeast extract, 0.1% $MgSO_4$, pH 7.0; 199, polyamine-free growth medium from Flow Lab., Irvine, U.K., pH 7.0.

[c]Harvested at logarithmic growth phase (others were harvested at stationary growth phase).

[d]Strain ATCC 3333[T] has been shown to be different from strain IFO 13615[T] (Pot, 1996).

vestigated. In regard to the 2-hydroxy fatty acids, all group 2 strains contained 2-hydroxy-octadecenoic acid ($C_{18:1\ 2OH}$) and/or 2-hydroxy-*iso*-nanodecenoic acid ($C_{19:0\ iso\ 2OH}$). Only one group 1 species with Q-8, namely *A. autotrophicum*, contained a single 2-hydroxy-fatty acid, namely undecanoic acid ($C_{11:0\ 2OH}$). *A. autotrophicum* is the only member of the genus known to be a facultative hydrogen autotroph, i.e., capable of growing with CO_2 as a sole carbon source under an atmosphere containing $H_2/O_2/CO_2$ (Aragno and Schlegel, 1978). Although hydrogen autotrophy may not have been tested in other species, the separate genotypic characteristics of this species (Gillis et al., unpublished results) support these aberrant chemotaxonomic and phenotypic characteristics and its separate taxonomic position among the aquaspirillae (see below sub taxonomic comments). Chemotaxonomic data on polyamines separated the *Aquaspirillum* species into six groups (Hamana et al., 1994). The species

A. metamorphum, *A. giesbergeri*, *A. anulus*, *A. delicatum*, *A. gracile*, *A. sinuosum*, *A. autotrophicum*, *A. putridiconchylium*, and *A. serpens* belong to the 2-hydroxyputrescine–putrescine type. *A. aquaticum* and *A. psychrophilum* belong to the 2-hydroxyputrescine–putrescine–spermidine type and differ from the above seven species by the presence of spermidine in addition to putrescine and 2-hydroxyputrescine, suggesting a corresponding phylogenetic difference. *A. dispar* belongs to the diaminopropane–2-hydroxyputrescine–putrescine–spermidine–cadaverine type. *A. peregrinum* belongs to the putrescine–spermidine type, *A. itersonii* to the putrescine–spermidine–homospermidine type, and *A. polymorphum* to the 2-hydroxyputrescine–putrescine–spermidine-homospermidine type. These last three species differ from the species mentioned above in their dominant polyamines; it has been shown (see below) that these species occupy a separate phylogenetic position.

TABLE BXII.β.83. Differential characteristics for species of the genus *Aquaspirillum* and comparison to related and former species of the genus[a]

Betaproteobacteria

Differential characteristics	*Spirillum volutans*	*Prolinoborus fasciculus*	*Comamonas terrigena* biovar *aquaticum*	*Aquaspirillum delicatum*	*Aquaspirillum gracile*	*Aquaspirillum metamorphum*	*Aquaspirillum psychrophilum*	*Aquaspirillum sinuosum*	*Aquaspirillum giesbergeri*	*Aquaspirillum anulus*	*Aquaspirillum itersonii* subsp. *nipponicum* (ATCC 33333)	*Aquaspirillum serpens* subsp. *serpens*	*Aquaspirillum serpens* subsp. *bengal*	*Aquaspirillum arcticum*[b]	*Aquaspirillum autotrophicum*
Cell diameter, μm[c]	1.4–1.7	0.7–0.9	0.5–0.6	0.3–0.4	0.2–0.3	0.7–1.3	0.7–0.9	0.6–0.9	0.7–1.4	0.8–1.4	0.5–0.8	0.6–1.1	0.9–1.2	1.0	0.6–0.8
Cell morphology	H	SR	H	V	H	H	H	H	H	H	H	H	H	SR-V	H
Type of helix[d]			C		C	C	C	C	C	C	CC	C	C		C
Wavelength of helix, μm[e]	16–28		2.0–5.0	–	2.8–3.5	7.5–12.0	5.5–6.5	8.6–10.5	4.5–8.4	5.0–13.0	2.5–6.0	3.5–12.0	4.6–8.1		3.0–4.0
Helix diameter, μm[e]	5.0–8.0		0.8–1.0	0.4–0.7	0.5–2.1	2.2–3.5	1.0–1.4	1.4–3.5	1.2–5.0	1.7–4.5	1.2–2.0	1.2–4.2	1.7–2.3		
Length of helix, μm[e]	14–60	3.6–43.0	2.5–13.0	3.0–5.0	3.5–14.0	3.5–11.0	1.5–14.0	5.0–42.0	4.0–40.0	4.0–52.0	2.0–10.0	3.5–42.0	5.2–22.0	3.0–7.0	2.0–5.0
Polar membrane present	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Poly-β-hydroxybutyrate formed	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+
Type of flagellation	BT	BT	BT	U(1–2)	BT	BT	BT	BT	BT	BT	BT	BT	BT	BS	BT
Coccoid bodies dominant after 3–4 weeks	–	+	–	+	–	–	–	–	–	–	+	–	–	+	–
Acid produced from sugars[f]	–	–	–	–	+	–	–	–	–	–	+	–	–	+	–
Temperature range for growth in °C			12–42	09–40	10–42	03–38	02–26	09–37	09–36	3–36	12–42	12–44	15–42	0–20	10–35
Optimal temperature for growth is 20°C and no growth at >26°C	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Optimal growth temperature is 5°C	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Optimal growth temperature is 41°C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Obligately microaerophilic	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease[g]	–	–	–	+	+	–	–	+	+	–	W	d	–	–	+
Indole test	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
Nitrate only reduced to nitrite	–	–	+	+	+	–	–	–	–	–	–	d	–	–	–
Anaerobic growth with nitrate	–	+	–	–	+	–	+	–	–	–	+	–	+	–	–
Denitrification	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–
Hydrolysis of esculin	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Hydrolysis of casein and starch	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Hydrolysis of hippurate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hydrolysis of gelatin at 30°C after 4 d	–	–	–	–	–	+	–	–	–	–	–	d	–	–	–

(continued)

Characteristic													
Liquefaction of gelatin at 20°C after:													
7 d	−	−	+	+	+	+	+	+	−	−	d	−	−
28 d	+	−	+	+	+	+	+	+	d	−	d	−	−
Growth factors required[h]	−	−	−	+	−	−	−	−	−	−	−	+	−
Glutamate used as sole carbon source	+	+	+	+	+	+	−	−	+	+	+	−	+
Histidine used as sole carbon source	−	−	−	−	−	−	−	+	+	−	−	−	−
Tryptophan and glycine used as sole carbon source	−	−	−	−	−	−	−	−	−	−	−	−	+
Nitrogenase activity[g]	+	−	+	+	−	−	−	−	d	−	−	−	−
Hydrogen autotrophy[g]	−	+	−	−	−	−	−	−	−	−	−	−	+
pH range for growth	5.5–8.5	5.5–9.0	5.5–8.5	6.0–9.0	5.5–9.0	6.0–9.0	6.0–9.0	6.0–8.5	5.5–9.0	6.0–9.0	6.0–8.4	5.5–7.6	5.0–8.0
Growth in the presence of:													
1% Oxgall	+	+	+	+	+	+	+	−	+	+	+	−	+
1% Glycine	−	−	−	−	−	−	−	+	−	−	−	−	−
3% NaCl	+	−	−	−	−	−	−	−	−	−	−	−	−
Water-soluble brown pigment formed in the presence of:													
0.1% tyrosine	−	−	−	−	−	−	−	−	−	−	+	−	−
0.1% tryptophan	−	−	−	+	−	−	−	−	−	−	+	−	−
Water-soluble yellow-green fluorescent pigment formed	W	+	−	d	−	−	−	−	−	d	−	−	−
Alkaline reaction in litmus milk	−	−	−	−	−	−	−	+	−	−	−	−	−
Growth on:													
Eosine methylene blue agar	+	+	+	+	+	+	−	+	+	+	−	−	−
MacConkey agar	+	+	+	−	+	+	−	+	d	d	−	−	−
Triple–Sugar–Iron agar	+	+	+	−	+	+	−	+	+	d	+	+	−
Sellers agar	+	+	+	−	+	+	−	+	d	d	+	−	−
Methyl red–Voges–Proskauer broth	+	+	+	−	−	−	−	−	+	−	−	−	−
Reduction of 0.3% H₂SeO₃	− or W	−	−	−	−	−	−	−	−	−	−	−	−
H₂S from 0.2% cysteine in PSS broth after 7 d	+	+	+	+	+	+	+	+	+	+	+	+	−
H₂S from 0.01% cystine in NA after 7 d	+	+	+	−	−	d	d	W	W	+	+	+	+
Deoxyribonuclease	−	+	−	+	+	+	+	−	+	d	d	−	+[j]
Ribonuclease	−	+	+	+	−	−	+	−	+	d	d	−	−
Mol% G + C of the DNA	38	64–65	63	64–65	65	57–59	57–58	58–59	66	49–51	52	NA	60–62

[a]Symbols: +, positive for all strains; −, negative for all strains; W, weak reaction; blank space, not determined; H, helical (one or more complete turns or twists); V, vibrioid (less than one complete turn or twist); SR, straight rod; C, clockwise helix; CC, counterclockwise helix; BT, bipolar tufts; U (1–2), 1 or 2 flagella at only one pole; BS, single flagellum at each pole; US, single flagellum at one pole; L, lophotrichous flagellation; d, differs among strains.

[b]The phylogenetic position of *A. arcticum* has not been investigated.

[c]By phase-contrast microscopy of 24- to 48-h-old broth cultures.

[d]Determined by focusing on the bottom of the cells. The pattern //// indicates a clockwise (right-handed) helix, whereas the pattern \\\\ indicates a counterclockwise (left-handed) helix.

[e]*Prolinoborus fasciculus* is a straight rod, the length of the helix refers to the length of the rod; *A. delicatum* is vibrioid rather than helical; length of helix can not be determined; helix diameter refers to the width of the vibrio; length of helix refers to the length of the vibrio.

[f]For *A. gracile*, acid from D-glucose, D-galactose, and L-arabinose (aerobically). For *Leuispirillum itersonii*, acid from glycerol (aerobically), fructose (aerobically and anaerobically), and glucose (anaerobically only). For *Leuispirillum peregrinum*, acid from fructose (aerobically and anaerobically). Peptone concentrations must be kept low (0.2% or less) in order to detect changes in pH indicator. Although *Leuispirillum itersonii* and *Leuispirillum peregrinum* acidify sugar media anaerobically, turbid growth does not occur and the organisms should be considered to have mainly a respiratory rather than fermentative type of metabolism. *A. arcticum* uses fructose, glucose, and ribose, although the use of fructose is listed positive as well as negative in the original publication.

[g]See Procedures for Testing Special Characters.

[h]*A. gracile* requires biotin and *Comamonas terrigena* biovar aquaticum requires niacin.

[i]Acid formation from D-glucose and D-fructose belong to the differentiating features for the discrimination of *Azospirillum* species.

[j]Positive at 37°C but not at 41°C.

(continued)

TABLE BXII.β.83. *(cont.)*

Differential characteristics	Betaproteobacteria			Alphaproteobacteria											
	Aquaspirillum putridiconchylium	*Aquaspirillum dispar*	*Aquaspirillum polymorphum*	*"Leisispirillum itersonii* subsp. *itersonii"*	*"Leisispirillum peregrinum* subsp. *peregrinum"*	*"Leisispirillum peregrinum* subsp. *integrum"*	*Magnetospirillum magnetotacticum*	*Oceanospirillum pusillum*	*Azospirillum brasilense*	*Azospirillum lipoferum*	*Azospirillum amazonense*	*Azospirillum halopraeferens*	*Azospirillum doebereinerae*	*Azospirillum irakense*	*Azospirillum largimobile*
Cell diameter, μm[c]	0.7–1.2	0.5–0.7	0.3–0.5	0.4–0.6	0.5–0.7	0.5–0.7	0.2–0.4	0.3–0.5	0.3–0.4	0.2–0.3	0.7–1.3	0.7–1.4	1.0–1.5	0.6–0.9	0.7–1.5
Cell morphology[d]	H	H	H	H	H	H	H	H	V	H	H	H	H	H	H
Type of helix[d]	C	C	CC	CC	CC	CC	C	CC	C	C	C				
Wavelength of helix, μm[e]	4.5–7.0	2.0–3.5	4.0–5.0	2.5–6.0	3.0–4.5	3.0–4.5	1.0–2.0	1.7–2.0	—	2.8–3.5	7.5–12.0	5.5–6.5			
Helix diameter, μm[e]	1.2–2.0	1.0–2.1	1.0–1.5	1.0–2.2	1.4–2.0	1.4–2.2		1.0–1.2	0.4–0.7	0.5–2.1	2.2–3.5	1.0–1.4			
Length of helix, μm[e]	4.0–23.0	2.1–6.5	3.5–8.4	2.0–10.0	1.5–22.0	1.5–22.0	4.0–6.0	1.2–4.0	3.0–5.0	3.5–14.0	3.5–11.0	1.5–14.0			
Polar membrane present	+	+	+	+	+	+	+	+	+	+	+	—	+	+	
Poly-β-hydroxybutyrate formed	+	—	+	+	+	+	+	+	+	+	+			+	
Type of flagellation	BT	BT	BS	BT	BT	BT	BS	BS	U(1–2)	BT	BT	BT	US	US + L	US + L
Coccoid bodies dominant after 3–4 weeks	—	—	+	+	+	—	+	—	—	—	—	—	+	+	
Acid produced from sugars[f]	—	—	—	+	+	+	—	—	—	+	—	—	d[i]	—	+
Temperature range for growth in °C	8–40	10–44	14–36	12–42	11–40	11–40		11–39	09–40		03–38	02–26	25–37	30–43	14–43.5
Optimal temperature for growth is 20°C and no growth at >26°C	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—
Optimal growth temperature is 5°C	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Optimal growth temperature is 41°C	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Obligately microaerophilic	—	—	—	—	—	—	+	—	+	+	+	+	+		
Oxidase	+	+	+	+	+	+	—	W or –	+	+	+	+	+		W
Catalase	+	+	+	+	+	+	—	W	+	+	+	—	+		W
Phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+		
Urease[g]	—	—	+	—	+	+	+	—	+	+	—	+	—		—
Indole test	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nitrate only reduced to nitrite	—	—	—	—	—	—	—	—	—	+	—	+	—	d	—
Anaerobic growth with nitrate	—	+	—	+	—	—	+	—	—	—	—	+	—	—	—
Denitrification	—	+	+	+	+	+	+	—	+	+	—	+	+	—	+
Hydrolysis of esculin	—	—	+	+	+	+	—	—	—	—	—	—	—	—	—
Hydrolysis of casein and starch	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hydrolysis of hippurate	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hydrolysis of gelatin at 30°C after 4 d	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

(continued)

Liquefaction of gelatin at 20°C after:
- 7 d
- 28 d

Growth factors required[h]

Glutamate used as sole carbon source

Histidine used as sole carbon source

Tryptophan and glycine used as sole carbon source

Nitrogenase activity[g]

Hydrogen autotrophy[g]

pH range for growth — 5.5–8.5, 6.0–8.5, 5.5–9.0, 5.5–9.0, 5.5–9.0, 5.5–8.5, 6.0–9.0, 6.0–9.0, 6.8–8, 5.5–8.5

Growth in the presence of:
- 1% Oxgall
- 1% Glycine
- 3% NaCl

Water-soluble brown pigment formed in the presence of:
- 0.1% tyrosine
- 0.1% tryptophan

Water-soluble yellow-green fluorescent pigment formed

Alkaline reaction in litmus milk

Growth on:
- Eosine methylene blue agar
- MacConkey agar
- Triple–Sugar–Iron agar
- Sellers agar

Methyl red–Voges–Proskauer broth

Reduction of 0.3% H_2SeO_3

H_2S from 0.2% cysteine in PSS broth after 7 d

H_2S from 0.01% cystine in NA after 7 d

Deoxyribonuclease

Ribonuclease

Mol% G + C of the DNA — 52, 63–65, 61–62, 60–64, 60–62, 64, 65, 51, 63, 64–65, 63, 69–70, 69.6, 64–67, 70

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[a] Symbols: +, positive for all strains; −, negative for all strains; W, weak reaction; blank space, not determined; H, helical (one or more complete turns or twists); V, vibrioid (less than one complete turn or twist); SR, straight rod; C, clockwise helix; CC, counterclockwise helix; BT, bipolar tufts; U (1–2), 1 or 2 flagella at only one pole; BS, single flagellum at one pole; US, single flagellum at each pole; L, lophotrichous flagellation; d, differs among strains.

[b] The phylogenetic position of *A. arcticum* has not been investigated.

[c] By phase-contrast microscopy of 24- to 48-h-old broth cultures.

[d] Determined by focusing on the bottom of the cells. The pattern //// indicates a clockwise (right-handed) helix, whereas the pattern \\\\ indicates a counterclockwise (left-handed) helix.

[e] *Prolinoborus fasciculus* is a straight rod, the length of the helix refers to the length of the rod; *A. delicatum* is vibrioid rather than helical; length of helix can not be determined; helix diameter refers to the width of the vibrio; length of helix refers to the length of the vibrio.

[f] For *A. gracile*, acid from D-glucose, D-galactose, and L-arabinose (aerobically). For *Levispirillum itersonii*, acid from glycerol (aerobically), fructose (aerobically and anaerobically), and glucose (anaerobically only). For *Levispirillum peregrinum*, acid from fructose (aerobically and anaerobically). Peptone concentrations must be kept low (0.2% or less) in order to detect changes in pH indicator. Although *Levispirillum itersonii* and *Levispirillum peregrinum* acidify sugar media anaerobically, turbid growth does not occur and the organisms should be considered to have mainly a respiratory rather than fermentative type of metabolism. *A. arcticum* uses fructose, glucose, and ribose, although the use of fructose is listed positive as well as negative in the original publication.

[g] See Procedures for Testing Special Characters.

[h] *A. gracile* requires biotin and *Comamonas terrigena* biovar *aquaticum* requires niacin.

[i] Acid formation from D-glucose and D-fructose belong to the differentiating features for the discrimination of *Azospirillum* species.

[j] Positive at 37°C but not at 41°C.

ENRICHMENT AND ISOLATION PROCEDURES

A number of enrichment methods have been used, usually taking advantage of the ability of aquaspirillae to grow with levels of nutrients low enough to discourage active growth of many other organisms. Two methods employed by Williams and Rittenberg (1957) have yielded excellent results:

a. Peptone or yeast autolysate (1%) is added to a sample of the source water. The samples are incubated at room temperature for ~1 week or until the spirillae become numerous. A portion of this culture is then added to an equal quantity of the source water and the mixture is sterilized by autoclaving. It is then inoculated from the unsterilized portion of the initial culture. After 1–3 transfers through successively nutrient-exhausted medium, the spirillae predominate.

b. A second method is to enrich the initial sample of source water with 1% calcium malate or lactate and incubate for ~1 week. A serial transfer is then made into more source water similarly supplemented with malate or lactate. Spirillae predominate after 3 or 4 such transfers.

For isolation, the enrichments are diluted 1:100 to 1:100,000 with sterile tap water. The dilution bottles are shaken vigorously and allowed to stand at room temperature for 20 min to allow migration of spirillae to the surface of the diluent. Isolation is then accomplished by streaking the surface water onto a suitable medium such as PSS agar or nutrient agar.

For enrichment by the use of putrid infusions of mussels or mud and sand samples, see Terasaki (1963, 1970, 1980); Jannasch (1965) summarized other general methods. Special methods have been used for the following organisms: *A. gracile*, see Canale-Parola et al. (1966); *A. autotrophicum*, see Aragno and Schlegel (1978); *A. bengal*, see Kumar et al. (1974).

MAINTENANCE PROCEDURES

Aquaspirillae may be maintained in semisolid PSS medium at 30°C (except for *A. psychrophilum*, which is maintained at 15°C) with weekly transfer (Hylemon et al., 1973b). Cultures may also be maintained as nutrient agar stabs at room temperature (except for *A. psychrophilum* which is maintained in a refrigerator) with monthly transfer (Terasaki, 1972).

Preservation is most easily accomplished by adding a dense suspension of cells to nutrient broth containing 10% (v/v) dimethylsulfoxide, with subsequent freezing in liquid nitrogen. Terasaki (1975) reported a method for freeze-drying spirillae.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Characterization methods for aquaspirillae have been described in detail by Terasaki (1972, 1979) and by Hylemon et al. (1973b). The following comments refer to certain aspects of these procedures. Cell dimensions are best measured in wet mounts of broth cultures by phase-contrast microscopy, rather than by dark-field microscopy or by light microscopy of stained smears. To determine whether the cells have a clockwise or counter-clockwise type of helix, see footnote d of Table BXII.β.83. The presence of intracellular poly-β-hydroxybutyrate is best determined by chemical analysis; for example, *A. delicatum* has no visible granules but does make the polymer. The type and number of flagella is best determined by electron microscopy rather than by flagella staining (Williams, 1960). With regard to coccoid bodies, all strains have a few such forms in old cultures; however, it is only in certain species that coccoid bodies become predomi-

nant in old cultures and have taxonomic significance. For testing the acidification of sugar media it is important to use a low concentration of peptone (0.2% or less). For the urease test, cells should be cultured in PSS broth for 24 h and centrifuged and suspended in sterile water to a dense concentration. An aliquot of 0.5 ml of this suspension is then added to 2.0 ml of a medium consisting of 0.1% BES buffer (*N,N*-bis (2-hydroxyethyl)-2-aminoethane sulfonic acid), 2% urea, and 0.001% phenol red; pH 7.0. This medium must be sterilized by filtration because of the thermolability of the urea. A red or magenta color after incubation at 30°C for 24 h indicates a positive reaction, provided that controls in similar media lacking urea remain colorless. For detection of a water-soluble fluorescent pigment, cultures are streaked in a line across plates of PSS agar and incubated for 48–72 h; the covers of the plates are removed and the plates examined with an ultraviolet lamp of the type used for mineralogical specimens (254-nm wavelength). The occurrence of a distinct, yellow-green, fluorescent zone in the agar medium surrounding the growth constitutes a positive test. Cultures to be tested for nitrogenase activity should be cultured in nitrogen-deficient semisolid malate medium (see the genus *Azospirillum*) supplemented with 0.005% yeast extract. Cultures are incubated for 3 d at 30°C and then sealed with rubber vaccine bottle stoppers. Acetylene is injected to a final concentration of 10% (v/v) and the cultures are tested for ethylene production by gas chromatography after 1 h of further incubation. Controls using liquid rather than semisolid medium, and semisolid medium containing 0.1% $(NH_4)_2SO_4$, should be negative for ethylene production. For testing hydrogen autotrophy, the mineral medium of Aragno and Schlegel (1978) is used[2]. Cultures are incubated under an atmosphere of $O_2/CO_2/H_2$ (5:10:85). A requirement for both H_2 and CO_2 should be demonstrated. For testing sole carbon sources, the procedures of Terasaki (1972, 1979) or Hylemon et al. (1973b) should be followed for most species (see Tables BXII.β.79 and BXII.β.83 for additional methods). It is recommended that the type strain of the suspected species be subjected to the same battery of characterization tests as used for the new isolate in order to confirm an identification. Chemotaxonomic characteristics of the aquaspirillae and *Magnetospirillum* can be tested as described by Sakane and Yokota (1994) and Hamana et al. (1994).

DIFFERENTIATION OF THE GENUS *AQUASPIRILLUM* FROM OTHER GENERA

Table BXII.β.84 indicates the characteristics of *Aquaspirillum* that distinguish it from other genera with similar morphological or physiological features. The phylogenetic diversity observed is used to delineate the different subgroups considered within the genus *Aquaspirillum*. The renamed *Aquaspirillum* species (see below) have been represented by their new generic names.

TAXONOMIC COMMENTS

In the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Krieg, 1974), a single genus, *Spirillum*, contained all of the

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2. Mineral medium (g/l): $Na_2HPO_4 \cdot 12H_2O$, 9.0; KH_2PO_4, 1.5; $MgSO_4 \cdot 7H_2O$, 0.2; NH_4Cl, 1.0; ferric ammonium citrate, 0.005; $CaCl_2 \cdot 2H_2O$, 0.01; R trace element solution (see below), 3.0 ml; pH 7.1. For a solid medium, 17.0 g of agar is added. For both liquid and solid media, 0.05% $NaHCO_3$ should be incorporated aseptically into the sterilized medium to buffer against changes in pH caused by the CO_2 of the gas atmosphere. Trace element solution (mg/l): $ZnSO_4 \cdot 7H_2O$, 10.0; $MnCl_2 \cdot 4H_2O$, 3.0; H_3BO_3, 30.0; $CoCl_2 \cdot 6H_2O$, 20.0; $CuCl_2 \cdot 6H_2O$, 0.79; $NiCl_2 \cdot 6H_2O$, 2.0; $Na_2MoO_4 \cdot 2H_2 0$, 3.0.

TABLE BXII.β.84. Differential characteristics of the genus *Aquaspirillum* and other genera of oxidase-positive, motile, curved, vibrioid, or helical Gram-negative rods[a]

Differential characteristics	*Aquaspirillum*	*Spirillum*	*Oceanospirillum*	*Campylobacter*	*Azospirillum*	*Bdellovibrio*	*Pseudomonas*	*Vibrio*	*Alteromonas*	*Prolinoborus*	*Comamonas*	*Levispirillum*	*Herbaspirillum*	*Marinospirillum*
Predominant cell shape:														
Helical	+[b]	+	+	−[c]	+	−	−	−	−	−	−	+	+	+
Vibrioid or curved	−[b]	−	−	+[c]	+[d]	+	−[e]	d	d	+	+	−	+	−
Straight rod	−[b]	−	−	−	−[d]	−	+[e]	d	d	−	+	−	−	−
Cell diameter, μm[b]	0.2–1.4	1.4–1.7	0.3–1.4	0.2–0.5	1.0	0.2–0.5	0.5–1.0	0.5–0.8	0.7–1.5	0.7–0.9	0.3–0.8	0.4–0.8	0.6–0.7	0.3–1.2
Polar membrane present	+	+	+	+	+	−	−	−	−	+	d	+	−	+
Unusual arrangement of polar flagella:														
Bipolar tufts	+[f]	+	+[g]	−	−	−	−	−[h]	−	+	+	+	+	+
Tuft at one pole	−[f]	−	−[g]	−	−	−	d	+[h]	+	−	+	−	+	−
Single flagellum at one or both poles	−[f]	−	−[g]	+	+	+	d	d	−	−	−	−	−	−
Lateral flagella also formed under certain conditions	−	−	−	−	+	−	d	d	−	−	−	−	−	−
Intracellular poly-β-hydroxybutyrate formed	+[i]	+	+	−	+	−	d	−	−	+	+	+	+	+
Relation to oxygen:														
Aerobic	+	−	+	−	+	+	+	+	+	+	−	+[m]	+	d
Facultative	−	−	−	+	−	−	−	+	−	−	−	−[m]	+	d
Microaerophilic	−	+	−	+	−	−	−	−	−	−	−	−[m]	−	d
CO₂ required for growth	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Seawater or Na⁺ required for growth	−	−	+	−	−	d	d	+[j]	+	−	−	−	−	+
Tolerant to 3% NaCl	−[k]	−	+	d	d	d		+	+	−	d[l]	−[l]	+	+
Indole test	−	−	−	−	−	−	d	d	+	−	−	d[l]	+	−[n]
Carbohydrates are catabolized	−	−	−	−	+	d	d	+	−	+	+	−	+	−
Habitat:														
Freshwater	+	+	−	−	−	d	d	d	+	+	+	+	+	−
Marine	−	−	+	−	−	d	d	d	+	−	+	−	−	+[o]
Soil	−	−	−	+	+	d	d	d	−	−	+	−	+	−
Humans or mammals	−	−	−	+	−	+	−	−	−	−	−	−	−	−
Capable of multiplying in the periplasmic space of other bacteria	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Nitrogenase activity	−	−	−		+	−	−	−	−	−	−	d[l]	d[l]	−
Mol% G + C	49–66	38	42–51	30–38	69–71	33–37, 42–51	57–70	38–51	38–50	62–65	60–69	60–66	60–65	42–45

[a] Symbols: +, all species positive except where noted; −, all species negative except where noted; d, differs among species.

[b] *A. delicatum* is mainly vibrioid.

[c] Cells in chains resemble spirilla.

[d] In pure cultures, a proportion of the cells may be straight rods.

[e] The genus *Pseudomonas* contains straight rods and rods that are curved in one plane, but not helically curved rods.

[f] *A. delicatum* mainly has a single flagellum at one pole; *A. polymorphum* mainly has a single flagellum at each pole.

[g] *Oceanospirillum pusillum* mainly has a single polar flagellum at each pole.

[h] Most species of *Vibrio* have a single polar flagellum, but two species (*V. fischeri* and *V. logei*) have a tuft of polar flagella.

[i] *A. gracile* and *A. psychrophilum* lack this polymer.

[j] Growth of all species is stimulated by NaCl, and most species have an absolute requirement for Na⁺.

[k] *A. gracile*, *Levispirillum itersonii*, and *Levispirillum peregrinum* can catabolize a very restricted number of sugars. All other species are incapable of catabolizing any carbohydrates.

[l] Only a few carbohydrates are used.

[m] Some species fix nitrogen in microaerophilic conditions, but with a source of fixed nitrogen they grow as aerobes.

[n] Only known for *M. minutulum*.

[o] *M. megaterium* has been isolated from kusaya gravy (used to produce traditional dried fish).

various aerobic and microaerophilic spirillae. However, the DNA base composition for this genus ranged from 38 to 65 mol% G + C, a range much greater than expected for a well-defined bacterial genus. Within the genus, three groups became evident: (a) the aerobic freshwater spirillae that could not tolerate 3% NaCl (mol% G + C is 49–66); (b) the aerobic marine spirillae that required seawater for growth (mol% G + C is 42–51); and (c) the large microaerophilic spirillae that belonged to the species *Spirillum volutans* (mol% G + C is 38). Accordingly, Hylemon et al. (1973a, b) divided the genus into three genera *Aquaspirillum*, *Oceanospirillum*, and *Spirillum*, respectively.

In the first edition of the *Bergey's Manual of Systematic Bacteriology* (Krieg, 1984a), for practical purposes, the single genus *Aquaspirillum* was maintained for a considerably heterogeneous group of microorganisms, as indicated by the wide range of mol% G + C (49–66). Phenotypically, however, the genus *Aquaspirillum* was based largely on a pattern of core properties considered typical for the genus. These included, besides the lack of tolerance to 3% NaCl: a helical shape; bipolar tufts of flagella; poly-β-hydroxybutyrate formation; a strictly respiratory type of metabolism; positive oxidase, catalase, and phosphatase reactions; an inability to attack sugars; hydrolysis of starch and casein; a negative indole test; an optimal growth temperature of 30°C; and a simple chemoheterotrophic nutrition with amino acids or the salts of organic acids serving as carbon sources. Species were assigned to the genus based on a similarity between their characteristics and this pattern of core features, with the recognition that exceptional characteristics may occur. Such exceptional characteristics are: a vibrioid shape or a straight rod shape, nitrogen-fixing ability, hydrogen autotrophy, lack of poly-β-hydroxybutyrate, high or low temperature optima for growth, a single flagellum at one or both poles, catabolism of a limited variety of sugars, and vitamin requirements.

Other than the wide mol% G + C range of the DNA, genotypic evidence to support the lack of phylogenetic relationship at the genus level of the various species of *Aquaspirillum* was scarce, and mainly based on 16S rRNA oligonucleotide catalogs of three *Aquaspirillum* species (Woese et al., 1982). *A. serpens* was shown to belong to group II of the phototrophic bacteria as defined by Gibson et al. (1979), or to the β-subdivision of the purple bacteria (*Proteobacteria*) (Woese et al., 1984c; Vandenberghe et al., 1985). It was found to be related to *Rhodospirillum tenue* and, to a lesser extent, to *Spirillum volutans*. *A. gracile* was also shown to be a member of group II, but was more closely related to *Rhodopseudomonas gelatinosa* than to *A. serpens*, *S. volutans*, or *R. tenue*. Importantly, *A. itersonii* was found to be a member of group I or the α-subdivision of the purple bacteria (*Proteobacteria*) (Woese et al., 1984b) and to be related to *Rhodospirillum rubrum* and *Azospirillum brasilense*. The relationship of *A. itersonii* and *A. polymorphum* to the genus *Azospirillum* was also demonstrated by rRNA–DNA hybridization studies (De Smedt et al., 1980).

Subsequent rRNA–DNA hybridizations (Willems et al., 1991a, c; Pot et al., 1992b; Pot, 1996), DNA–DNA hybridizations (Boivin et al., 1985; Pot, 1996), and 16S rRNA sequencing results (Kawasaki et al., 1997; Wen et al., 1999) confirmed this extensive genotypic heterogeneity and revealed some unexpected relationships. It is striking that most of these genotypic variations could not be linked to clear phenotypic descriptions of well-defined separate taxa. Still, some formal taxonomic changes have recently been introduced.

As suggested by Krieg (1984a), DNA–DNA hybridization showed that indeed some species did not deserve separate species status; e.g., it was shown that *A. serpens* and *A. bengal* belonged to a single species (Boivin et al., 1985; Pot, 1996), with DNA–DNA hybridization values between 59 and 73%. Consequently, *A. bengal* was included in *A. serpens* as *A. serpens* biovar bengal. *A. serpens* biovar bengal showed an average of 58% DNA–DNA relatedness to three reference strains of *A. serpens*, while the average DNA–DNA relatedness between these *A. serpens* strains (ATCC 12638^T, 15278, and 27050) was 72%. Considering these values and the clear phenotypic differences (Tables BXII.β.79 and BXII.β.83), *A. serpens* biovar bengal could be regarded as a subspecies of *A. serpens* or even as a separate species. The description of the two subspecies, *A. serpens* subsp. *bengal* and *A. serpens* subsp. *serpens*, was first mentioned by Pot (1996) and will be proposed here.

The position of *A. serpens* biovar azotum is, at present, not very clear. Pot (1996) showed by SDS-PAGE of whole-cell proteins that strain ATCC 11335 belonged to *A. serpens* (comparison with strains Murray VHA, Murray St. Rhodes, Murray VHL, Murray VH, ATCC 27050, and ATCC 12638^T), confirming some of the serological relationships observed by McElroy and Krieg (1972) and the phenotypic similarity found by Hylemon et al. (1973b) and Carney et al. (1975). However, strain ATCC 11335 was shown by SDS-PAGE of whole-cell proteins to be different from its supposed homologous subculture NCIB 9011 (Pot, 1996). The latter strain was found to be as far removed from *A. serpens* as the genera *Neisseria* and *Chromobacterium* and had no significant DNA–DNA relatedness with the other *A. serpens* strains (Pot, 1996). Possibly this subculture of NCIB 9011 can be regarded as a contaminant.

A polyphasic study resulted in the inclusion of *A. aquaticum* and 13 clinical isolates previously designated EF group 10 (Falsen, 1996) in *Comamonas terrigena* (Willems et al., 1991c; and the chapter on *Comamonas* in this *Manual*). Consequently *A. aquaticum* should be considered as a junior synonym of *Comamonas terrigena*. The synonymy between *C. terrigena* and *A. aquaticum* was previously suggested by 16S rRNA cataloging data (Woese et al., 1984c).

Seven other *Aquaspirillum* species were classified in the *Comamonadaceae* based on rRNA–DNA hybridizations and 16S rDNA sequencing (Willems et al., 1991c; Wen et al., 1999; the chapter on the *Comamonadaceae* in this *Manual*): *A. anulus*, *A. delicatum*, *A. giesbergeri*, *A. gracile*, *A. metamorphum*, *A. sinuosum*, and *A. psychrophilum*. These species are phylogenetically too distantly removed from the type species, *A. serpens*, to be considered members of the genus *Aquaspirillum*. *A. anulus*, *A. giesbergeri*, and *A. sinuosum* share a relatively low mol% G + C content (57–58). SDS-PAGE analysis of whole-cell proteins showed that the type strains of *A. giesbergeri* and *A. sinuosum* are very similar (a Pearson correlation of 0.9), with only small differences in the zone with molecular weights between 40,000 and 70,000, and therefore possibly belong to a single species. Further DNA–DNA hybridization studies are necessary to confirm this similarity. *A. giesbergeri* and *A. sinuosum* together constitute a separate rRNA branch in the *Comamonadaceae*. The position of *A. anulus* as a separate rRNA branch has been solely determined by rRNA–DNA hybridizations (Pot et al., 1992b).

A. delicatum, *A. gracile*, *A. metamorphum*, and *A. psychrophilum* have mol% G + C values of 62–66. *A. psychrophilum* and *A. metamorphum* have 97.2% 16S rDNA sequence similarity and con-

stitute a separate rRNA branch in the *Comamonadaceae. A. delicatum* belongs to the same rRNA branch as *Rhodoferax* (96.8% 16S rDNA sequence similarity). More data are needed to determine whether *A. delicatum* should be considered as a third species in *Rhodoferax* (Hiraishi et al., 1991a; Hiraishi, 1994; Madigan et al., 2000b), which currently contains two species of curved rods to spirillae, able to grow phototrophically and having a mean mol% G + C of ~61. A possible relationship between *A. delicatum* and *Comamonas* had already been suggested (Krieg, 1984a) because *A. delicatum* is vibrioid rather than helical, and has one or two flagella at only one pole rather than bipolar flagellar tufts. Although Leifson (1962) placed the organism in the genus *Spirillum* (*Aquaspirillum*), he recognized that it differed morphologically from typical spirillae and suggested the possibility of creating a new genus for such non-carbohydrate-utilizing vibrios.

A. gracile belongs to a separate group deeply branching within the *Comamonadaceae*. The detailed chemotaxonomic data on polyamines, quinones, 2- and 3-hydroxy fatty acids, and main nonpolar fatty acids (Hamana et al., 1994; Sakane and Yokota, 1994) are displayed in Tables BXII.β.80, BXII.β.81, and BXII.β.82, but do not completely support the division on the basis of 16S rDNA sequence analysis. For example, *A. psychrophilum* differs considerably from *A. metamorphum* in its polyamine content and in its 3-hydroxy fatty acid content.

The considerable phenotypic inertness of the aquaspirillae, the lack of uniform phenotypic testing procedures, and the large phenotypic differences observed with the other genera of the *Comamonadaceae* make it difficult to phenotypically describe *Aquaspirillum* species within the family *Comamonadaceae*. Until more phenotypic characters are available, or until genotypic parameters can be used exclusively for the description of taxonomic entities, the species will be maintained in the genus *Aquaspirillum*.

Aquaspirillum fasciculus received special attention in the first edition of *Bergey's Manual of Systematic Bacteriology* (Krieg, 1984a). This species showed many characteristics atypical for the aquaspirillae (straight-rod shape, forming of viscous flocs, failing to swim except in a viscous medium, and nitrogenase activity). However, *A. fasciculus* did possess bipolar tufts of flagella, formed poly-β-hydroxybutyrate, had a strictly respiratory type of metabolism, did not attack sugars, could not grow with 3% NaCl (or even 1% NaCl), had a simple nutrition, grew best at 30°C, was indole-negative, did not hydrolyze starch or casein, possessed oxidase, catalase, and phosphatase activity, and had a mol% G + C content of the DNA of 62–65. Moreover, *A. fasciculus* formed coccoid bodies in abundance, which is a characteristic of certain aquaspirillae and other helical or vibrioid bacteria (*Oceanospirillum*, *Campylobacter*, *Desulfovibrio*, and *Vibrio*; see Williams and Rittenberg, 1957; Ogg, 1962; Levin and Vaughn, 1968; Felter et al., 1969; Baker and Park, 1975). Furthermore, the presence of a polar membrane is also a characteristic mainly associated with helical bacteria (Murray and Birch-Andersen, 1963; Hickman and Frenkel, 1965a, b; Keeler et al., 1966; Ritchie et al., 1966), although it has also been found in the rod-shaped organism *Chromatium* (Murray and Birch-Andersen, 1963). Nitrogenase activity, typical for *A. fasciculus*, but unusual for aquaspirillae, did occur in two helical species. The morphology of some aquaspirillae is variable: Strength et al. (1976) reported the isolation of a strain with rods very similar to *A. fasciculus*, but after prolonged transfer many cells were found to be curved or even S-shaped. Moreover, some typical helical species develop variants

that are nearly straight rods upon prolonged transfer (Williams, 1959a, b; Terasaki, 1973). Genotypic comparison of *A. fasciculus* to other *Aquaspirillum* species by rRNA–DNA hybridization showed that the species has to be regarded as belonging to a separate genus, for which the name *Prolinoborus* has been proposed (see the genus *Prolinoborus* in this family).

Another species with a separate phylogenetic position is *A. autotrophicum*, which can grow autotrophically under an atmosphere of oxygen, carbon dioxide, and hydrogen. Other members of *Aquaspirillum* are not facultative hydrogen autotrophs or have not been tested for this character. The other characteristics of *A. autotrophicum* are completely consistent with the description of the genus *Aquaspirillum*. However, rRNA–DNA hybridization data showed that this species is phylogenetically more closely related to *Janthinobacterium lividum* and *Herbaspirillum* than to *A. serpens*, the type species of the genus *Aquaspirillum* (Pot, 1996). 16S rDNA sequence analysis confirmed the relationship between *Herbaspirillum* and *Janthinobacterium* (Kirchhof et al., 2001). Whether *A. autotrophicum* can be considered as a separate species of *Herbaspirillum*, or deserves a separate genus status, requires further study.

The taxonomic position of *A. dispar* has been partially clarified. The two available strains of *A. dispar* (ATCC 27510^T and ATCC 27650) showed 88–100% DNA–DNA binding, and have nearly identical electrophoretic protein profiles (Aragno and Schlegel, 1978; Pot, 1996). Both strains shared the highest rRNA cistron similarity with *Chromobacterium violaceum* NCTC 9757^T, although according to 16S rRNA cataloging, *A. dispar* appeared to be more closely related to *A. serpens* than to *Chromobacterium violaceum*. Because of a $T_{m(e)}$ difference of 6°C (Pot, 1996) or more, and some considerable phenotypic differences, *A. dispar* cannot be assigned to the genus *Chromobacterium*. More data are required to determine its exact relationship to a new genus, *Vogesella* (see the genus *Vogesella* in this family), which is phylogenetically the closest neighbor of *Chromobacterium*.

A. putridiconchylium also belongs in the *Betaproteobacteria*, where it constitutes a separate branch (Pot, 1996); a close taxonomic relationship with *A. dispar* is excluded. Because neither of these species can be discriminated phenotypically from the *Aquaspirillum* species, we continue to consider them as separate species of the genus *Aquaspirillum*.

Three species of the genus *Aquaspirillum* belong to the *Alphaproteobacteria* based on rRNA–DNA hybridization and 16S rRNA gene sequencing. *A. itersonii* and *A. peregrinum* were more closely related to *Rhodospirillum rubrum* and *Rhodospirillum photometricum* (Kawasaki et al., 1997), and formed a separate branch in the *Alphaproteobacteria*. *A. polymorphum* was shown to be most closely related to *Magnetospirillum gryphiswaldense*. Schleifer et al. (1991) proposed this generic name for the former species *Aquaspirillum magnetotacticum* (Blakemore et al., 1979, 1989) and the new isolate *M. gryphiswaldense*. Also, it was shown by rRNA–DNA hybridization that *A. itersonii* and *A. peregrinum* form a separate rRNA branch at the node of the *Azospirillum* and *Rhodospirillum rubrum* branch (Pot, 1996). The different subspecies of *A. peregrinum* and *A. itersonii* were shown by 16S rRNA gene sequence comparison to be very highly related within each species (Kawasaki et al., 1997). *A. peregrinum* subsp. *peregrinum* and *A. peregrinum* subsp. *integrum* showed 91% DNA–DNA relatedness and clearly belong to a single species (Pot, 1996). In our hands, however, the two subspecies of *A. itersonii* were not closely related. *A. itersonii* subsp.

itersonii was situated on the *A. peregrinum* rRNA branch, but *A. itersonii* subsp. *nipponicum* was found to belong to the *Betaproteobacteria* (A. Willems unpublished results). In these studies, different subcultures of the type strain were used (*Aquaspirillum itersonii* subsp. *nipponicum* ATCC 33333[T] by Pot (1996) and *Aquaspirillum itersonii* subsp. *nipponicum* IFO 13615[T] by Kawasaki et al., 1997).

In contrast to the aquaspirillae of the *Betaproteobacteria*, the aquaspirillae of the *Alphaproteobacteria* all contain a counter-clockwise type of helix and can hydrolyze esculin. Using these characteristics, they can easily be discriminated from the authentic aquaspirillae and from *Prolinoborus*. Given these genotypic and phenotypic differences, it is possible to allocate two of these species to a new genus. The name *Levispirillum*, with *L. itersonii* and *L. peregrinum*, has been proposed (Pot, 1996) and has been effectively published but not yet validly published. The position of *A. polymorphum* is not clear: phenotypically it could belong to *Levispirillum*, but genotypically it definitely belongs to the genus *Magnetospirillum* (Kawasaki et al., 1997). It certainly does not belong phylogenetically to *Aquaspirillum*. A formal transfer, however, has not been proposed, probably because of the extensive phenotypic differences between *A. polymorphum* and *Magnetospirillum*.

Although the genus *Oceanospirillum* belongs to the *Gammaproteobacteria*, *O. pusillum* has been shown to belong to the *Alphaproteobacteria* (Woese et al., 1985; Kawasaki et al., 1997) by 16S rDNA sequence analysis. This position was confirmed by rRNA–DNA hybridization results (Pot, 1996). Using either method, the type strain occupies a separate branch in the *Alphaproteobacteria*. Although this species also has a counter-clockwise type of helix, is not capable of esculin hydrolysis, requires 3% NaCl for growth, and occupies a phylogenetically distinct position, its inclusion in the genus *Levispirillum* is questionable. Kawasaki et al. (1997) suggested creation of a new genus for this species.

Butler et al. (1989) isolated and described a new psychrophilic, straight-to-curved rod-shaped bacterium, with an optimal growth temperature of 5°C, from an Arctic sediment. It was assigned to the genus *Aquaspirillum* as *Aquaspirillum arcticum* based on its morphological and physiological characteristics. It was distinguished from *A. psychrophilum* (Terasaki, 1973, 1979) by a different optimal temperature for growth (5°C rather than 20°C), and by its ability to grow at 0°C. Unlike *A. psychrophilum*, *A. arcticum* will also grow in vitamin-free, defined, basal salts–glucose medium. Other biochemical characteristics are listed in Table BXII.β.83. *A. arcticum* was found to acquire thermotolerance when heat shocked or treated with nalidixic acid, two conditions responsible for the introduction of heat shock- or stress proteins (McCallum and Inniss, 1990; Gounot, 1991). This indicates that growth temperature is not necessarily a reliable parameter for delineation of species. Genotypic or phylogenetic information is not presently available for *A. arcticum*.

Taxonomic conclusions   In the past, species descriptions have been based mainly on differences in morphology, nutrition, and DNA base composition. In some cases, such species are obviously different; for example, a small spirillum such as *A. polymorphum* would not belong to the same species as a large spirillum (such as *A. serpens*), and a spirillum having a mol% G + C of 50 would not be placed in the same species as one with a mol% G + C of 60. However, species distinctions within a particular morphological group of strains having a similar DNA base composition are less firm. Another difficulty is that many species are represented by only one or two strains, and the limits of variation within such a species may be broader than is presently assumed. Therefore, it was suggested by Krieg (1984a) that some species may not deserve separate species status while other species may represent more than a single species. Genotypic analysis of most aquaspirillae confirmed these observations. The true aquaspirillae should be restricted to the type species *Aquaspirillum serpens*, including the former species *Aquaspirillum bengal*. All other species are phylogenetically too distantly related to be regarded as "true" or authentic aquaspirillae. However, with the exception of a few species, the majority cannot be described reliably from the phenotypic point of view. Therefore they are presently retained in the genus *Aquaspirillum*. The genus definition needed only minor adaptations so as to compensate for the formal removal of *Aquaspirillum fasciculus*, *Aquaspirillum aquaticum*, *Aquaspirillum peregrinum*, and *Aquaspirillum itersonii*.

The two *species incertae sedis*, "*Spirillum minus*" and "*Spirillum pulli*", do not belong to the genera *Aquaspirillum*, *Oceanospirillum*, or *Spirillum*, and their placement is uncertain. Studies of these species have been hampered by the lack of reproducible *in vitro* cultivation methods. They do not appear on the Approved Lists of Bacterial Names because no type or reference strains are available and the organisms are not well characterized. The disease syndromes caused by these species are distinct and recognizable, however. If possible, neotype strains should be designated and either maintained by animal passage or preserved in a recognized culture collection.

FURTHER READING

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Pot, B., M. Gillis and J. De Ley. 1992. The genus *Aquaspirillum. In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd Ed., Vol. 4, Springer-Verlag, New York. pp. 2569–2582.

List of species of the genus Aquaspirillum

1. **Aquaspirillum serpens** (Müller 1786) Hylemon, Wells, Krieg and Jannasch 1973b, 366[AL] (*"Vibrio serpens"* Müller 1786, 48.)
   ser'pens. L. v. *serpo* to crawl or creep; L. part. adj. *serpens* creeping.

The morphological characters are depicted in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 35°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources

are listed in Table BXII.β.79; the best growth occurs with glutamate. Nitrate is not used as a sole nitrogen source; results with ammonium salts have been conflicting (Terasaki, 1972; Hylemon et al., 1973b). A defined medium suitable for batch and continuous cultures has been described by Whitby and Murray (1980).

Habitat: pond waters.

Aquaspirillum serpens belongs phylogenetically to the *Betaproteobacteria* (Pot, 1996). The former species *Aquaspirillum bengal* (Kumar et al., 1974, 457) has been assigned to *A. serpens* as *A. serpens* biovar bengal (Boivin et al., 1985). Based on sufficient phenotypic differences and a low level of DNA relatedness, *A. serpens* biovar bengal could be regarded as a subspecies of *A. serpens* or even as a separate species.

The mol% G + C of the DNA is: 49–51 (T_m).

Type strain: ATCC 12638, DSM 68.

a. **Aquaspirillum serpens** subsp. **serpens** subsp. nov. (*Aquaspirillum serpens* (Müller 1786) Hylemon, Wells, Krieg and Jannasch 1973b, 366; "*Vibrio serpens*" Müller 1786, 48.)

Cell diameter 0.6–1.1 μm. Description as for the species. No brown pigments in media with aromatic components; some strains do produce water-soluble fluorescent pigments. Growth at temperatures from 12 to 44°C with 35°C as optimum. Can grow at pH values up to 9. Some strains produce gelatinase and ribonuclease. Can grow on EMB agar. KNO_3 can sometimes be reduced to KNO_2 (*Aquaspirillum serpens* subsp. *serpens* var. azotum ATCC 11335).

The mol% G + C of the DNA is: 49–51 (T_m).

Type strain: ATCC 12368, DSM 68.

b. **Aquaspirillum serpens** subsp. **bengal** subsp. nov. (*Aquaspirillum bengal* Kumar, Banerjee, Bowdre, McElroy and Krieg 1974, 457.)

ben′ gal. M.L. n. *bengal* Bengal.

The description is as for the species, but the cells are usually shorter. They have a diameter of 0.9–2.2 μm, the wavelength of the helix is 4.6–8.1 μm, and the helix length ranges from 5.2 to 22 μm. Brown pigments can be formed on media containing 0.1% tyrosine or tryptophan. Water-soluble fluorescent pigments are not produced. Can grow at temperatures from 12 to 42°C; 41°C is the optimal growth temperature. Can grow at pH values up to 8.4. No growth on EMB agar, no gelatinase and ribonuclease activity. Prefer to grow in microaerophilic conditions. KNO_3 can not be reduced to KNO_2.

The mol% G + C of the DNA is: 52 (T_m).

Type strain: ATCC 27641.

2. **Aquaspirillum anulus** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973b, 368[AL] (*Spirillum anulus* Williams and Rittenberg 1957, 86.)

a′ nu.lus. L. masc. n. *anulus* a ring.

The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 27–30°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources are listed in Table BXII.β.79. Ammonium salts can be used as sole nitrogen sources; nitrate is not used.

Isolated from pond water and from putrid infusions of fresh water mussels.

Aquaspirillum anulus belongs phylogenetically to the *Comamonadaceae* (Willems et al., 1991c; Wen et al. 1999; Willems and Gillis, The *Comamonadaceae*, this volume) in which it constitutes a separate branch.

The mol% G + C of the DNA is: 58–59 (T_m).

Type strain: ATCC 35958, NCIB 9012.

3. **Aquaspirillum arcticum** Butler, McCallum, Inniss 1990, 320[VP] (Effective publication: Butler, McCallum, Inniss 1989, 265.)

arc′ ti.cum. L. adj. *arcticum* of the Arctic, where the species was first isolated.

The morphological characters are described in Table BXII.β.83. The species is atypical in that it possesses only a single polar flagellum rather than bipolar tufts. The species is considered psychrophilic (defined as organisms having an optimal temperature for growth of ~15°C or lower, a maximum temperature for growth of ~20°C, and a minimum growth temperature of 0°C or below; (Morita, 1975). Optimal growth temperature is 5°C. Differential characteristics are described in Table BXII.β.83. The species is atypical in that it produces acid from carbohydrates (fructose, glucose, and ribose).

Isolated from Arctic sediment; other habitats unknown.

There are no phylogenetic data available for *Aquaspirillum arcticum*.

The mol% G + C of the DNA is: not reported.

Type strain: Res-10, ATCC 49402, DSM 6444.

4. **Aquaspirillum autotrophicum** Aragno and Schlegel 1978, 116[AL]

au.to.tro′ phi.cum. Gr. n. *autos* self; Gr. adj. *trophikos* nursing, tending or feeding; M.L. neut. adj. *autotrophicum* self-nursing or self-feeding.

The morphological characters are described in Table BXII.β.83. Optimal growth temperature 28°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources are listed in Table BXII.β.79. Ammonium salts and nitrate can be used as sole nitrogen sources.

Aquaspirillum autotrophicum is phylogenetically not closely related to the type species of *Aquaspirillum* (Pot, 1996), but belongs to the *Herbaspirillum–Janthinobacterium* rDNA branch (Gillis et al., 1991; Baldani et al., 1996; Willems et al., unpublished results). Further studies are required to determine whether it belongs in *Herbaspirillum* and/or if it deserves a separate genus status.

Isolated from an eutrophic lake in Switzerland.

The mol% G + C of the DNA is: 60–62 (T_m).

Type strain: SA 32, ATCC 29984, DSM 732.

5. **Aquaspirillum delicatum** (Leifson 1962) Hylemon, Wells, Krieg and Jannasch 1973b, 371[AL] (*Spirillum delicatum* Leifson 1962, 164.)

de.li.ca′ tum. L. neut. adj. *delicatum* delicate.

Vibrioid. Chains of cells may resemble spirillae. The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83, and are most characteristic for

cultures grown in nutrient broth rather than PSS broth. Intracellular poly-β-hydroxybutyrate granules are not evident, but chemical tests indicate the presence of this polymer. Optimal growth temperature 30–32°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources are listed in Table BXII.β.79. Growth is poor in defined media; the best growth occurs with malate as the carbon source and glutamine as the nitrogen source. Ammonium salts or potassium nitrate are used poorly as nitrogen sources.

Aquaspirillum delicatum belongs phylogenetically to the *Comamonadaceae* (Willems et al., 1991c; Wen et al. 1999; Willems and Gillis, The *Comamonadaceae*, this volume).

Isolated from stored distilled water.

The mol% G + C of the DNA is: 63 (T_m).

Type strain: 146, ATCC 14667, CCUG 15846, DSM 11558, LMG 4328, NCIB 9419.

GenBank accession number (16S rRNA): AF078756.

6. **Aquaspirillum dispar** Hylemon, Wells, Krieg and Jannasch 1973b, 372[AL]

dis'par. L. neut. adj. *dispar* unlike.

The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 30°C. Moderate growth at 25 and 37°C; no growth at 10 or 45°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Although originally described as being unable to grow anaerobically with nitrate (Hylemon et al., 1973b), the species does grow well under these conditions (Aragno and Schlegel, 1978). Sole carbon sources are listed in Table BXII.β.79. Ammonium salts are used well as sole nitrogen sources; nitrate is not used.

Isolated from fresh water.

Aquaspirillum dispar is phylogenetically not closely related to the type species of *Aquaspirillum* (Pot, 1996); because more than 84% DNA–DNA hybridization was found with the type strain of *Microvirgula aerodenitrificans* (Cleenwerck et al., 2003), both names can be considered as subjective synonyms.

The mol% G + C of the DNA is: 63–65 (T_m).

Type strain: ATCC 27510, DSM 736.

7. **Aquaspirillum giesbergeri** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973b, 368[AL] (*Spirillum giesbergeri* Williams and Rittenberg 1957, 88.)

gies'ber.ger.i. M.L. n. *giesbergeri* of Giesberger, the first investigator to study certain physiological characteristics of spirillae.

The morphological characters are depicted in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 30°C. Chemotaxonomic and physiological characters are as described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources are listed in Table BXII.β.79. Ammonium salts can be used as sole nitrogen sources; nitrate is not used.

This species includes organisms previously assigned to the two species *"Spirillum giesbergeri"* and *"Spirillum graniferum"* by Williams and Rittenberg (1957). The two species were combined into a single species by Hylemon et al.

(1973b) based on a high degree of similarity in phenotypic characters and in DNA base composition. *Aquaspirillum giesbergeri* belongs phylogenetically to the *Comamonadaceae* (Willems et al., 1991c; Wen et al., 1999; Willems and Gillis, The *Comamonadaceae*, this volume) where it constitutes a separate rRNA branch together with *A. sinuosum*. By SDS-PAGE of whole-cell proteins, it was shown that the type strains of *A. giesbergeri* and *A. sinuosum* are very similar (a correlation of 0.9) and therefore possibly belong to a single species. Further DNA–DNA hybridization data are necessary to confirm this homology.

Isolated from pond water.

The mol% G + C of the DNA is: 57–58 (T_m).

Type strain: ATCC 11334, DSM 9157, NCIB 9073, NRRL B-2060.

8. **Aquaspirillum gracile** (Canale-Parola, Rosenthal and Kupfer 1966) Hylemon, Wells, Krieg and Jannasch 1973b, 369[AL] (*Spirillum gracile* Canale-Parola, Rosenthal and Kupfer 1966, 124.)

gra.ci'le. L. neut. adj. *gracile* slender or thin.

The smallest of the aquaspirillae. The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83. When originally isolated, all strains formed subsurface, spreading, semitransparent colonies on a medium containing 1.0% agar, the spreading occurring within the medium (Canale-Parola et al., 1966). After prolonged subculturing, some of the spirillae in each strain lost the ability to diffuse through 1.0% agar and formed small, nonspreading colonies. *A. gracile* was originally described as being microaerophilic, based on the growth of the organisms a few millimeters below the surface of semisolid media (Canale-Parola et al., 1966). However, later analysis of the type strain indicated that in liquid media a maximum growth response occurred under an air atmosphere (Laughon, 1973). Optimal growth temperature 30°C. Scanty growth in PSS broth at 25°C; no growth at 10 or 42°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Carbon sources are listed in Table BXII.β.79. Ammonium chloride can be used as a nitrogen source but potassium nitrate cannot (Canale-Parola et al., 1966). A chemically defined medium has been devised by Laughon (1973; see also Krieg, 1976). Biotin is required for growth. Isolation is accomplished by allowing the spirilla to pass through a membrane filter disk (0.45 μm pore size) to an underlying agar medium (Canale-Parola et al., 1966).

Aquaspirillum gracile phylogenetically belongs to the *Comamonadaceae* (Willems et al., 1991c; Wen et al., 1999; see also Willems and Gillis in the chapter in this book on *Comamonas*).

Isolated from pond or stream water.

The mol% G + C of the DNA is: 64–65 (T_m).

Type strain: D4, ATCC 19624, DSM 9158.

GenBank accession number (16S rRNA): AF078753.

Additional Remarks: Reference strains are ATCC 19625 and 19626.

9. **Aquaspirillum metamorphum** (Terasaki 1961b) Hylemon, Wells, Krieg and Jannasch 1973b, 366[AL] (*Spirillum metamorphum* Terasaki 1961b, 220.)

me.ta.mor′phum. Gr. neut. adj. *metamorphum* changing.

The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 30°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources are listed in Table BXII.β.79. Ammonium salts can be used as a sole nitrogen source; nitrate is not used.

Isolated from the putrid infusion of a freshwater mussel.

Aquaspirillum metamorphum belongs phylogenetically to the *Comamonadaceae* (Willems et al., 1991c; Wen et al., 1999; Willems and Gillis, The *Comamonadaceae*). With *A. psychrophilum*, it constitutes a separate rRNA branch within this family.

The mol% G + C of the DNA is: 63 (T_m).

Type strain: ATCC 15280, CCUG 13794, DSM 1837, IFO 12012, LMG 4339, NCIB 9509.

GenBank accession number (16S rRNA): AF078757.

10. **Aquaspirillum polymorphum** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973b, 371[AL] (*Spirillum polymorphum* Williams and Rittenberg 1957, 85.)
po.ly.mor′phum. Gr. adj. *poly* many; Gr. n. *morphus* form, shape; M.L. neut. adj. *polymorphum* of many shapes.

Although originally reported to have bipolar tufts of flagella (Williams and Rittenberg, 1957), this species appears to have only a single flagellum at each pole (Terasaki, 1972; Hylemon et al., 1973b). The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 30°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Although originally reported to grow anaerobically with nitrate (Williams and Rittenberg, 1957), more recent studies have not confirmed this result (Terasaki, 1972; Hylemon et al., 1973b). Sole carbon sources are listed in Table BXII.β.79. The best sole carbon sources are glutamate and aspartate; they are also the best sole nitrogen sources. Ammonium salts can serve as nitrogen sources; there are conflicting results concerning the utilization of nitrate as a sole nitrogen source (Terasaki, 1972; Hylemon et al., 1973b). Growth in PSS broth abundant, cloudy. Colonies on PSS agar are circular, convex, translucent, pinpoint.

Aquaspirillum polymorphum is a member of the *Alphaproteobacteria*; it is possibly most closely related to the genus *Magnetospirillum* (Pot, 1996; Kawasaki et al., 1997).

Isolated from pond water.

The mol% G + C of the DNA is: 61–62 (T_m).

Type strain: ATCC 11332, DSM 9160, IAM 14441, IFO 13961, NCIB 9072, NRRL B-2066.

11. **Aquaspirillum psychrophilum** (Terasaki 1973) Terasaki 1979, 138[AL] (*Spirillum psychrophilum* Terasaki 1973, 57.)
psy.chro′phi.lum. Gr. adj. *psychros* cold; Gr. adj. *philus* liking, preferring; M.L. neut. adj. *psychrophilum* preferring cold.

The morphological characters are described in Table BXII.β.83. Grows in nutrient broth or PSS broth. Optimal growth temperature 20°C. No growth above 26°C. The organism may be best described as psychrotolerant or psychrotrophic rather than psychrophilic (Morita, 1975). Che-

motaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. This species has not been cultured in vitamin-free defined media and most likely has a growth factor requirement.

Isolated from Antarctic mosses.

Aquaspirillum psychrophilum belongs phylogenetically to the *Comamonadaceae* (Willems et al., 1991c, Wen et al., 1999; Willems and Gillis, The *Comamonadaceae*, this volume). With *A. metamorphum*, it constitutes a separate rRNA branch within this family.

The mol% G + C of the DNA is: 65 (T_m).

Type strain: CA 1, ATCC 33335, DSM 11588, IFO 13611, LMG 5408.

GenBank accession number (16S rRNA): AF078755.

12. **Aquaspirillum putridiconchylium** (Terasaki 1961a) Hylemon, Wells, Krieg and Jannasch 1973b, 367[AL] (*Spirillum putridiconchylium* Terasaki 1961a, 80.)
pu′ tri.di.con.chy.li.um. L. adj. *putridus* putrid, decayed; L. n. *conchylium* a shellfish; L. n. *putridiconchylium* decayed shellfish.

The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 32°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources are listed in Table BXII.β.79. Ammonium salts can be used as a sole nitrogen source; nitrate is not used.

Isolated from the putrid infusion of a freshwater mussel.

Phylogenetically, *Aquaspirillum putridiconchylium* is not closely related to the type species of *Aquaspirillum* (Pot, 1996). It constitutes a separate branch in the *Betaproteobacteria*.

The mol% G + C of the DNA is: 52 (T_m).

Type strain: ATCC 15279, IFO 12013, NCIB 9508.

13. **Aquaspirillum sinuosum** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973b, 368[AL] (*Spirillum sinuosum* Williams and Rittenberg 1957, 94.)
si.nu.o′ sum. L. neut adj. *sinuosum* full of curves.

The morphological characters are shown in Fig. BXII.β.67 and described in Table BXII.β.83. Optimal growth temperature 30°C. Chemotaxonomic and physiological characters are described in Tables BXII.β.80, BXII.β.81, BXII.β.82, and BXII.β.83. Sole carbon sources are listed in Table BXII.β.79. Ammonium salts can be used as a sole nitrogen source; nitrate is not used.

Aquaspirillum sinuosum phylogenetically belongs to the *Comamonadaceae* (Willems et al., 1991c; Wen et al., 1999; Willems and Gillis, The *Comamonadaceae*, this volume); it constitutes a separate rRNA branch together with *A. giesbergeri*. By SDS-PAGE of whole-cell proteins, it was shown that the type strains of *A. sinuosum* and *A. giesbergeri* are very similar (a correlation of 0.9) and therefore possibly belong to a single species. Further DNA–DNA hybridization studies are necessary for confirmation.

Isolated from freshwater.

The mol% G + C of the DNA is: 57–59 (T_m).

Type strain: ATCC 9786, CCUG 13728, LMG 4393, NCIB 9010, NCMB 59, NRRL B-2065, VPI 18.

GenBank accession number (16S rRNA): AF078754.

Other Organisms

1. *"Aquaspirillum bipunctata"* Dubinina, Grabovich, Lysenko, Chernykh and Churikova 1993.

bi.punc.ta' ta. L. *bis* twice; L. part. adj. *punctatus* punctate, dotted; M.L. fem. adj. *bipunctata* twice punctate.

Dubinina et al. (1993) discussed the diagnostic criteria of the genus *Thiospira* and the taxonomic validity of this genus. Six strains of gigantic sulfur spirillae, whose characteristics corresponded to the species diagnosis of *Thiospira winogradski* and *"Thiospira bipunctata"*, were shown to belong to the genera *Spirillum* and *Aquaspirillum* according to phenotypic properties, nucleotide composition, and DNA relatedness studies. Strains D-405, D-409, D-410, D-411, and D-423, which were morphologically similar to *"Thiospira bipunctata"* formed a tight cluster phenotypically and genotypically and were assigned to a new species within the genus *Aquaspirillum* as *"Aquaspirillum bipunctata"* comb. nov. They are isolated from sediments of sewage from aerotanks and a water pond containing up to 7 mg/l H_2S. Strain D-427, which corresponded to the description of *Thiospira winogradski*, was proposed as a species of the genus *Spirillum*. The species name has not been validly published.

The mol% G + C of the DNA is: 48–51 (T_m).

Deposited strain: D-411.

2. *"Aquaspirillum denitrificans"* Dubinina, Churikova, Grabovich, Chernykh, Raichenstein and Petukhova 1989.

de.ni.tri' fi.cans. M.L. adj. *denitrificans* pertaining to the property of denitrification.

Spiral-shaped cells, from 1 to 1.4 μm wide, 2–4.1 to 25–30 μm long. Motile with bipolar flagella. Gram negative. Poly-β-hydroxybutyrate, volutin, and elemental sulfur (in the presence of sulfides) are accumulated in the cells. Colonies are flat, nonpigmented, 2–4 mm in diameter. Facultatively anaerobic growth with nitrates, fumarate, or molecular oxygen as electron acceptors. Under anaerobic conditions, nitrates are reduced to molecular nitrogen. Growth within a pH range of 6.0–8.5; optimal pH is 7.0–8.2. Optimal temperature for growth is 28°C. Chemoorganotrophs, using a wide variety of organic acids for growth: acetate, succinate, malate, fumarate, benzoate, α-ketoglutarate, oxaloacetate, pyruvate, lactate, and glyoxylate. Use several amino acids as carbon source: tyrosine, proline, asparagine, and cysteine. Growth on yeast extract and casein hydrolysate; vitamin solution is essential for growth. Does not use carbohydrates and alcohols. Nitrates, ammonium salts, casein hydrolysate, yeast extract, peptones, cysteine, and serine are used as nitrogen source. Catalase, oxidase, and urease positive. Casein and starch not hydrolyzed. Nongelatinolytic. Does not produce indole. Nitrate reduced to nitrites. Hydrogen sulfide produced from cysteine. Does not grow with 3% NaCl. Forms brown products on benzoate medium. Cannot fix nitrogen and does not contain proteolytic enzymes. Two very similar strains have been isolated from the sewage sludge of a putrefying aero tank. A limited phenotypic comparison with *A. metamorphum, A. giesbergeri, A. itersonii,* and *A. anulus* has been published. The species name has not been validly published.

The mol% G + C of the DNA is: 60 (T_m).

Deposited strain: D-415.

3. *"Aquaspirillum elegans"* Grabovich, Churikova, Chernykh, Leshcheva, Pushkina, Shipilova and Panteleyeva 1990.

e' le.gans. L. adj. *elegans* elegant.

Helical cells with pointed ends. Cell diameter varies from 0.6 to 1.0 μm. The length of the helix is 6.5–11 μm. Motile with bipolar flagella. Poly-β-hydroxybutyrate, volutin, and elemental sulfur (in the presence of sulfides) are stored in the cells. Colonies are flat, nonpigmented, 2–4 mm in diameter. Facultatively anaerobic growth with a strictly respiratory type of metabolism. Under anaerobic conditions, nitrates are reduced to nitrites. Grows in pH range 6.5–8.5; optimal pH is 7.5. Optimal temperature for growth is 28°C. Chemoorganotrophs, using a wide variety of organic compounds for growth: organic acids (acetate, succinate, malate, fumarate, citrate, isocitrate, oxaloacetate, aconitate, α-ketoglutarate, pyruvate, lactate), amino acids (lysine, tyrosine, proline, histidine, alanine, aspartate, and ornithine), alcohols (ethanol, glycerol, mannitol), and carbohydrates (fructose and sorbose), as well as yeast extract and casein hydrolysate. Vitamins and trace elements are essential for growth. Of the nitrogen sources investigated, growth occurred on nitrates, nitrites, ammonium salts, casein hydrolysate, yeast extract, peptone, aspartate, glutamate, cysteine, and serine. Nitrate reduced to nitrite. Casein, gelatin, and starch not hydrolyzed. Catalase, oxidase, and urease positive. Does not produce indole. Hydrogen sulfide produced, from cysteine. Does not grow with 3% NaCl. Isolated from sewage of purification installations. A limited phenotypic comparison with *A. peregrinum, A. dispar, A. itersonii, A. autotrophicum, A. sinuosum,* and *A. putridiconchylium* has been published. The species name has not been validly published.

The mol% G + C of the DNA is: 61.6 (T_m).

Deposited strain: D-425.

4. *"Spirillum pleomorphum"* Inoue and Komagata 1976, 170.

ple.o.mor' phum. L. adj. *pleomorphum* pleomorphic.

Helical cells, curved rods, crescent-shaped cells, U-form cells, and nearly ring-like forms occur on peptone–yeast extract–glucose (PYG) agar. Cell size: 0.7–1.0 × 2.0–4.5 μm. Motile by a single polar flagellum. Growth in PYG broth is turbid with sediment. Colonies on PYG agar are circular, smooth, convex, entire, opaque, and pale brown. Optimal growth temperature, 9°C; maximum, 20°C; minimum below 0°C. Aerobic. Oxidase and catalase positive. Indole, Voges–Proskauer, and methyl red tests are negative. No growth with 5% NaCl. Acid but no gas from xylose (aerobically). No acid or gas from glucose, lactose, sucrose, maltose, arabinose, or glycerol aerobically or anaerobically. No change in litmus milk. H_2S is not produced. Starch and cellulose are not degraded. Nitrate is reduced to nitrite. No growth occurs anaerobically with nitrate. Succinate, formate, acetate, fumarate, and propionate are assimilated. Citrate, lactate, protocatechuate, *p*-hydroxybenzoate, and hippurate are not assimilated. Isolated from Antarctic soil. Differences from *A. arcticum* include its high number of pleomorphic cells, its ability to reduce nitrate, its production of acid from xylose but not from glucose, its assimilation of formate but not lactate, and its higher temperature optimal for growth (9 versus 5°C).

The mol% G + C of the DNA is: 63 (T_m).
Deposited strain: 22-o-d, IAM 12028.

5. *"Aquaspirillum voronezhense"* Grabovich, Churikova, Chernykh, Kononykhina and Popravko 1987.

vo.ro.ne.zhen.se. M.L. adj. *voronezhense* pertaining to the city of Voronezh, former USSR.

Spiral-shaped cells, from 1.5–2.1 to 3 μm wide, with 1–3 turns of the spiral, 2.9–6.8 μm in diameter. Motile with bipolar flagella, in tufts of up to 50. Poly-β-hydroxybutyrate, volutin, and elemental sulfur (in the presence of sulfides) are stored in the cells. Colonies are flat, nonpigmented, 1–4 mm in diameter. Aerobic growth within a pH range of 6.0–9.0; optimal pH is 7.2–8.5. Optimal temperature for growth is 28°C. Chemoorganotrophs, using a wide variety of organic acids for growth including acetate, succinate, malate, fumarate, isocitrate, formate, α-ketoglutarate, oxaloacetate, pyruvate, salicylate, lactate, and glyoxylate. Strain D-420 differs from D-419 by its ability to use oxalate and aconitate and by the inability to grow on isocitrate and formate. Strain D-419 uses the amino acids proline, glycine, aspartate, glutamate, and valine; strain D-420 can use aspartate and tyrosine as carbon sources. A vitamin solution supplement is essential for growth. Ammonium salts, casein hydrolysate, yeast extract, peptone, aspartate, glutamate, cysteine, and serine (strain D-419) are used as nitrogen sources. Nitrate not reduced to nitrites. Casein and starch not hydrolyzed. Nitrates, sulfate, thiosulfate, and fumarate not used as electron acceptor. Catalase, oxidase, and urease positive. Hydrogen sulfide produced from cysteine. Do not produce indole. Form colored products on benzoate medium. The strains cannot fix nitrogen and do not contain proteolytic enzymes. Two very similar strains have been isolated from the sewage sludge of a putrifying aero tank. A limited number of DNA–DNA hybridization experiments showed that *"A. voronezhense"* is not related to *A. metamorphum*, *Levispirillum peregrinum*, and *"A. winogradskyi"*. The species name has not been validly published. Isolated from sewage sludge.

The mol% G + C of the DNA is: 58.5–60 (T_m).
Deposited strain: D-419.

Species Incertae Sedis

1. **"Spirillum minus"** Carter 1888, 47 (*Spirillum minor* (sic) Carter 1888, 47)*

mi'nus. L. neut. adj. *minus* less, smaller and refers to the small cell size.

Rigid cells; usually described as spiral with two or three turns, although the waves have been reported to be planar (McDermott, 1928). The ends of the cell may be blunt or pointed. Cell diameter, ~0.2 μm; cell length, 3–5 μm; wavelength, 0.8–1.0 μm. Actively motile by one or more flagella at each pole.

Causes one of the two forms of rat-bite fever in man. The disease caused by *"S. minus"* is often termed "Sodoku", it occurs worldwide but has its greatest frequency in the Far East. The organisms are usually transmitted to humans through the bite of an infected rat, although mice, squirrels, and rodent-ingesting animals such as cats, dogs, ferrets, and weasels have also been implicated. *"S. minus"* appears to be a natural parasite of rats, which act as carriers; the infection is usually not lethal in rats. The natural infection frequency for rats varies from country to country but may be as high as 25% (see Babudieri, 1973, for pertinent literature).

The clinical aspects of rat-bite fever and the distinctions between the form caused by *"S. minus"* and that caused by *Streptobacillus moniliformis* have been summarized by Joklik et al. (1980) and by Rogosa (1980). Experimental infections of humans and animals by *"S. minus"* have been described by Babudieri (1973).

"S. minus" is best observed in blood or exudates from patients by dark-field or phase-contrast microscopy of wet mounts; staining with Giemsa or Wright's stain or by silver impregnation is also useful.

"S. minus" is cultured *in vivo* by intraperitoneal inoculation of patients' blood or exudates from lesions, or blood from naturally infected rats injected into spirillum-free mice or guinea pigs (Rogosa, 1980); mice are the animals most susceptible to *"S. minus"* infection (Babudieri, 1973). It is questionable whether the organism has ever been cultured successfully in artificial media. Numerous attempts have failed, and various claims of successful cultivation have not been confirmed. One report that may indicate successful cultivation is that of Hitzig and Liebesman (1944), who inoculated blood from a patient into 2% dextrose–veal infusion broth and into 10% tomato extract–veal infusion broth. The addition of citrated human or rabbit blood was required for successful subculturing; also, the organisms initially required incubation in a candle jar but eventually were able to grow aerobically after five months of serial transfer. Confirmation of this report is needed. Considering the morphology, pathogenicity, and sources of *"S. minus"*, serious attention should be given to the possibility that the organism might belong to, or be related to, the genus *Campylobacter*, and the microaerophilic techniques employed for campylobacters might also prove useful for *"S. minus"*.

The species name has not been validly published. There are no type or reference strains.

The mol% G + C of the DNA is: not determined.

2. **"Spirillum pulli"** Mathey 1956, 745.

pul'li. L. gen. n. *pulli* of a young chicken.

Rigid spiral cells. By dark-field microscopy, the cell diameter is ~1 μm and the cell length is from 5 to 12 μm. Actively motile by means of a single flagellum at each end of the cell. Believed to be the cause of a diphtheroid stomatitis in the mouths of adult chickens. The lesions are yellowish white, rather firm, and adherent to the underlying tissue; often they are symmetrically ovoid, one at each side of the lower jaw. Lesions also occur on the palate, the lower surface of the tongue, the floor of the mouth, between the larynx and the transverse row of papillae on the tongue, around the larynx, and on the walls of the pharynx. The lesions vary in size from ~2 to 20 mm.

Attempts to culture *"S. pulli"* in artificial media have been unsuccessful. Experimental passage of the disease in chickens has been accomplished by contact and by experimental inoculation.

There are no type or reference strains. The species name has not been validly published.

The mol% G + C of the DNA is: not determined.

Editorial Note: The specific epithet *minor* is grammatically incorrect as noted by Robertson (1924) and the correct form is *minus*.

Genus IV. *Chromobacterium* Bergonzini 1881, 153[AL]

MONIQUE GILLIS AND NIALL A. LOGAN

Chro.mo.bac.te' ri.um. Gr. n. *chroma* color; Gr. n. *bakterion* a small rod; M.L. neut. n. *Chromobacterium* a small, colored rod.

Cells straight, round-ended, often coccoid, but usually occuring as rods, 0.6–0.9 × 1.5–3.0 μm, occurring singly, sometimes in pairs or short chains. No definite capsules are evident. No resting stages known. Gram negative. Usually contain poly-β-hydroxy-butyrate crystals (80% of strains positive), but rarely contain met-achromatic granules. **Motile by means of both a single polar flagellum and, usually, one or more subpolar or lateral flagella. Facultative anaerobes. Minimum temperature for growth 10–15°C; maximum about 40°C; optimal growth at 30–35°C.** Optimal pH 7–8; no growth occurs below pH 5. Grow on ordinary peptone media; no distinctive organic growth factor requirements. Colonies are smooth, but rough variants may occur; colonies are of butyrous consistency and are easily emulsified in water. **Most strains produce the violet pigment violacein**, but strains producing nonpigmented colonies are sometimes encountered, and subcultures of pigmented strains often contain partially or completely unpigmented colonies. Growth in nutrient broth produces a violet ring at the surface with a fragile pellicle. Chemoorganotrophs; **most strains (80%) attack carbohydrates fermentatively, some (20%) oxidatively, producing small amounts of acid** but usually no gas. Lactate is oxidized to CO_2. Usually oxidase positive by the method of Sivendra and Lo (1975). No growth in media containing 6% or more NaCl. Catalase positive, but very sensitive to hydrogen peroxide. Indole negative, Voges–Proskauer negative. Nitrate and nitrite reduced. Ammonia produced from peptone. Arginine hydrolyzed. **Hydrogen cyanide produced. Resistant to benzylpenicillin**, 10 mg/ml. **Sensitive to tetracycline**, 30 mg/ml. **Resistant to O/129 (2,4-diamino-6,7-diisopropyl pteridine)** by disc diffusion method, 50 mg/disc. Soil and water organisms, occasionally causing infections of humans and other mammals. 16S rRNA gene sequence data place *Chromobacterium* in the class *Betaproteobacteria*.

The mol% G + C of the DNA is: 65–68 (T_m).

Type species: **Chromobacterium violaceum** Bergonzini 1881, 153.

FURTHER DESCRIPTIVE INFORMATION

The pigment violacein is produced on or in media containing tryptophan. It is soluble in ethanol, but not in water or chloroform, and is readily identified by spectrophotometry and by testing with routine reagents (see Procedures for Testing for Special Characters). Subcultures of pigmented strains may contain partially or completely unpigmented colonies. An unpigmented strain is most readily recognized by having a colony morphology similar to that of a pigmented strain isolated at the same time under the same conditions. Once it has been confirmed as an oxidase- and catalase-positive, Gram-negative rod, such an isolate should be flagella-stained. If it possesses the characteristic polar and lateral flagella, it should then be subjected to the differential tests for the three genera *Chromobacterium, Iodobacter,* and *Janthinobacterium*. Although young cultures of *Aeromonas, Pseudomonas,* and *Vibrio* spp. grown on solid media sometimes produce lateral flagella in addition to their usual polar flagella, they give patterns of results different from those of the violet-pigmented organisms in the differential tests.

The characteristic flagellar arrangement is best seen in young cultures on solid media. The single polar flagellum is inserted at the tip of the cell, shows long, shallow waves, and often stains faintly. The lateral flagella are usually long. Usually 1–4 such flagella are produced, although up to eight may occur. They may be inserted subpolarly or laterally, usually show deep, short waves, and stain readily (see *Iodobacter fluviatilis* Fig. BXII.β.71).

Chromobacterium violaceum occurs in soil and water and is common in tropical climates. It occasionally causes serious pyogenic or septicemic infections of mammals, including humans (reviewed by Sneath, 1960; Gillis and De Ley,1992; with subsequent reports by Hassan et al., 1993; Ti et al., 1993; Roberts et al., 1997; Midani and Rathore, 1998; Desjardins et al., 1999). The disease can be cured by tetracyclines if treated at an early stage (Moss and Ryall, 1981) as well as by ciprofloxacin, norfloxacin, perfloxacin (Aldridge et al., 1988), gentamicin, imipenem, and trimethoprim–sulfamethazole (Midani and Rathore, 1998). In some cases, a sepsis mimicking melioidosis has been described (Chong and Lam, 1997), and potentially misidentification as *Burkholderia pseudomallei* might occur (Inglis et al., 1998). Pathogenic bacteria of carp have been characterized as closely resembling *Chromobacterium violaceum* (Rahmatullah and Beveridge, 1993; Kozinska and Antychowicz, 1996).

ENRICHMENT AND ISOLATION PROCEDURES

Selective and enrichment media have not been developed for *C. violaceum*, but colonies on routine growth media are readily recognized by their violet pigmentation, and isolates may be screened for the ability to grow at 37°C and for other characters that distinguish this organism from the species of *Iodobacter* and *Janthinobacterium* (Table BXII.β.85; see Procedures for Testing for Special Characters).

MAINTENANCE PROCEDURES

The organisms may survive for several years in dilute peptone water (0.1% peptone) at room temperature. They can also be preserved indefinitely by lyophilization or by freezing in nutrient broth containing 15% glycerol.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

To identify the pigment violacein, pigmented growth is shaken from a plate culture into a small volume of 96% (v/v) ethanol, and cells are then removed by centrifugation, followed by filtration through a 0.22 μm membrane; the solution shows an absorption maximum of 579 nm and a minimum of 431 nm if violacein is present. In 10% (v/v) H_2SO_4 in ethanol (96% v/v), the pigment gives a green solution with an absorption maximum at 699 nm and a minimum at 502 nm. Color reactions may be observed by mixing loopfuls of pigmented growth in a few drops of 96% (v/v) ethanol on a white tile; stirring in a drop of 25% (v/v) H_2SO_4 gives a green color, and stirring a drop of 10% (w/v) NaOH into a fresh growth/ethanol mixture gives first a green, then a reddish brown color. The biosynthesis of violacein has been studied intensively, and different metabolites have been characterized e.g., pseudodeoxyviolacein (Hoshino et al., 1994), deoxyviolacein, and chromopyrrolic acid (Hoshino et al., 1993a).

TABLE BXII.β.85. Patterns of results in tests useful for distinguishing *Chromobacterium* from *Iodobacter*, *Janthinobacterium*, and *Vogesella*

Characteristic	Percentage positive in species or group[a]				
	C. violaceum	*Iodobacter fluviatilis*	*J. lividum* (typical)	*J. lividum* (atypical)	*Vogesella*
Pigment:					
Indigoidin					+
Violacein	+	+	+	+	
Fluorescence[b]	− (T)[c]		− (T)[c]		100
Colonies on 1/4 NA:					
Spreading	0	83	0	0	
Gelatinous	0	4	36	100	
Tough	0	0	7	71	
Growth at:					
4°C	0	100	94	87	100
37°C	100	0	0	0	100
Anaerobic growth	100	100	3	0	
Nitrate reduction	87	98	84		100
Production of indole	0	0	0	0	100
Hydrolysis of Tween 80	+ (T)[c]		+ (T)[c]		0
Growth on:					
Citrate	100	85	95	94	0
Glucose:					
Fermented	10[d]	100	0	0[e]	0
Oxidized	0[d]	0	97	57[e]	0
Acid from:					
L-Arabinose[f]	0	0	100	87	0
D-Maltose	0	100	98	94	
myo-Inositol[g]	0	0	100	0	35
Trehalose	100	100	1	87	
Gelatinase	100	100	100	69	0
Lactate utilization	100	0	100	75	
Esculin hydrolysis	0	0	100	0	
Arginine hydrolysis	100	0	0	0	
Chitin digestion	100[h]		5		0
Caseinase	100	90	5	0	0
HCN production	100	0	0	0	

[a]From Logan (1989) with later amendments. Number of strains studied for each species: *C. violaceum*, 9; *Iodobacter fluviatilis*, 53; *J. lividum* (typical), 68; *J. lividum* (atypical), 14; *Vogesella*, 17.

[b]Fluorescence on chalk agar (Starr et al., 1960) with short wavelength illumination. All *Vogesella* strains exhibited very weak fluorescence.

[c]Only tested for the type strain; − (T), type strain negative; + (T), type strain positive.

[d]Occasional strains are oxidative.

[e]Some strains give no reaction in this test.

[f]Similar patterns of results are obtained with D-cellobiose and D-galactose.

[g]Similar patterns of results are obtained with D-sorbitol.

[h]Grimes et al. (1997) found that the type strain was negative under conditions different from those used by the authors.

L-Tryptophan is the carbon source for violacein, and isatin and indole 3-acetic acid are important metabolic intermediates in violacein production and can also serve as substrates. Violacein has trypanocide activity (Duran et al., 1994).

Because the production of violacein by *Chromobacterium violaceum* is dependent on *N*-acetylhomoserine lactones (AHLs), it provides a simple assay for the detection of these molecules, which are signal molecules in quorum sensing that interact with a transcriptional activator protein to couple gene expression to cell population density (McClean et al., 1997; Bachofen and Schenk, 1998).

The procedure of Mayfield and Inniss (1977) is recommended for studies of flagellar arrangement, and the even simpler technique of Heimbrook et al. (1989) appears, on limited experience, to be equally dependable. For the latter method, cultures are grown overnight on slopes of nutrient agar prepared at a quarter of the usual nutrient strength (1/4 NA), which have drops of condensate at their bases. A drop of the condensate is removed with a small loop and touched to a small drop of water on a clean slide.

The oxidase reactions of pigmented strains are commonly obscured by the pigment, but may be revealed by the method of Sivendra and Lo (1975). Smears of pigmented growth and control organisms are placed along the edge of a strip of filter paper that has been soaked in the reagent (1% [w/v] tetramethyl-*p*-phenylenediamine HCl) and supported on a glass slide. When the positive control has oxidized the reagent to a violet color, further drops of reagent are placed above the smears and the slide tilted so as to flush reacted reagent onto a white background.

Unless indicated otherwise, all media are autoclaved at 121°C for 15 min. Media are inoculated with one drop or loopful of quarter strength nutrient broth (1/4 NB) culture grown overnight at 25°C and, with the exception of those used for growth temperature tests, incubated at 25°C. This temperature permits strains of *Chromobacterium*, *Iodobacter*, and *Janthinobacterium* to grow and be compared in the differential tests. For assessment of growth temperatures, tubes of 1/4 NB are incubated in waterbaths at 37°C and 4°C and examined for growth at 2 and 7 d, respectively. To assess anaerobic growth, plates of nutrient agar (full strength) are streak-inoculated and incubated in an anaerobic jar (GasPak, BBL) for 7 d at 25°C.

To investigate oxidative and fermentative attack of glucose, the medium of Ward et al. (1986) is used without its H₂S indicator system. This modified medium contains (g/l): glucose, 10; casitone, 2; yeast extract, 1; phenol red, 0.04; bromothymol blue, 0.02; agar, 15. After heating to dissolve components and adjustment to pH 7.1, the medium is dispensed into tubes in 10-ml amounts, autoclaved, and left to set as deep-butted slopes, which are then inoculated by streaking the slope and stabbing the butt and incubated for up to 7 d. Fermentative organisms readily produce a yellow color throughout the medium, whereas a yellow or light green color restricted to the slope indicates oxidative attack. *Chromobacterium violaceum* usually attacks carbohydrates fermentatively without gas production, but aerogenic isolates have been reported (Sivendra, 1976), and as many as 20% of strains may show oxidative attack (Sneath, 1984a). *Iodobacter fluviatilis* is always fermentative and anaerogenic. Typical strains of *J. lividum* are oxidative, but atypical strains may give no reaction in this test.

Acid production from carbohydrates may be determined using a medium containing (g/l): (NH₄)₂HPO₄, 1; KCl, 0.2; MgSO₄·7H₂O, 0.2; yeast extract, 0.2; phenol red, 0.06; and agar, 12. After adjustment to pH 7.4 (orange), autoclaving, and addition of a filter-sterilized solution of L-arabinose, *myo*-inositol, D-maltose, D-mannitol, or trehalose to give a final concentration of 0.5%, the medium is dispensed into tubes and left to set as deep-butted slopes. Tubes are examined at 2, 4, and 7 d for acid (yellow) reactions in the slopes, butts, or both.

Utilization of lactate is assessed using a medium containing (g/l): lactic acid (sodium salt), 2; NaCl, 1; (NH₄)₂HPO₄, 1; KH₂PO₄, 0.5; MgSO₄·7H₂O, 0.2; phenol red, 0.06; agar, 12. After adjustment to pH 6.8 and autoclaving (115°C for 20 min), the medium is distributed into tubes and left to set as slopes. The medium is streak-inoculated and examined for alkaline (red) reactions at 2, 4, and 7 d.

For esculin hydrolysis, the medium of Sneath (1979) is used, which contains (g/l): peptone, 10; esculin, 1; sodium citrate, 1; ferric citrate, 0.05. After adjustment to pH 7.0, 5-ml quantities are dispensed in tubes and autoclaved. Tubes are examined at 3 and 4 d for browning.

Arginine-hydrolysis medium contains (g/l): L-arginine HCl, 10; NaCl, 5; peptone, 1; K₂HPO₄, 0.3; phenol red, 0.06; and agar, 3. It is adjusted to pH 7.2 (orange), dispensed in tubes to a depth of 2 cm, and autoclaved. Following stab-inoculation and sealing with sterile paraffin oil, tubes are incubated for 4 d and examined for alkaline (red) reactions.

Hydrogen cyanide indicator papers are prepared by dipping filter paper strips in saturated aqueous picric acid, drying (potentially explosive, care and doing under chemical hood recommended), and then dipping in 10% (w/v) aqueous sodium carbonate and drying again. A bottle of half-strength (semi-solid) nutrient agar is inoculated by stabbing and an indicator paper is trapped between the cap and rim; HCN production is indicated by the paper strip changing color from orange-yellow to brick-red within 3 d.

DIFFERENTIATION OF THE GENUS *CHROMOBACTERIUM* FROM OTHER GENERA

Table BXII.β.85 presents characteristics differentiating the genus *Chromobacterium* from the genera *Janthinobacterium, Iodobacter*, and *Vogesella. Microvirgula aerodenitrificans* is not included because comparable test results are not available.

TAXONOMIC COMMENTS

The present description of the genus *Chromobacterium* is based primarily on the description given by Sneath (1984a). The phylogenetic affiliation of *Chromobacterium* was studied first by DNA–rRNA hybridization (De Ley et al., 1978), and later by 16S rDNA sequence analysis. It is a member of the class *Betaproteobacteria*, in which it constitutes a separate lineage in the *Neisseriaceae*. Its closest neighbor is the recently proposed genus *Vogesella* (see the genus *Vogesella* in this family). *Vogesella* was created to contain the former *Pseudomonas indigofera* and new isolates of blue-pigmented bacteria isolated from fresh water bacterial communities. A single species, *Vogesella indigofera*, has been proposed because no DNA–DNA hybridizations between this species and the group of new isolates are available. *Vogesella* members do not contain violacein, but rather indigoidine. Also belonging to the *Neisseriaceae*, but more distantly related, is the genus *Iodobacter* (see Genus *Iodobacter*, this *Manual*). Earlier results from DNA–rRNA hybridization (Pot et al., 1992a) have shown that *Aquaspirillum dispar* and *Aquaspirillum putridiconchylium* also belong to this phylogenetic lineage. A new genus, *Microvirgula*, with the single species *Microvirgula aerodenitrificans* recently has been created for a denitrifying bacterium occupying a separate phylogenetic position between the *Vogesella–Chromobacterium violaceum* lineage and *Iodobacter fluviatilis* (Patureau et al., 1998). It is an aerobic as well as anoxenic heterotroph that has an atypical respiratory type of metabolism, in which oxygen and nitrogen oxides can be used simultaneously as terminal electron acceptors.

In addition to species of *Chromobacterium, Janthinobacterium*, and *Iodobacter*, which are found in soil and fresh water, violacein-producing organisms have been isolated from marine waters. Hamilton and Austin (1967) have named their strain "*C. marinum*", but Gauthier (1976) has considered his isolates to be excluded from *Chromobacterium* by their low mol% G + C range, and he assigned them to the genus *Alteromonas* as the new species *A. luteovioloaceus* (now *A. luteoviolacea*).

Species producing pigments that are not violacein, or even violet-colored in some cases, have at various times been assigned to *Chromobacterium*, and still occasionally appear in the literature as such. Of these, "*C. iodinum*" is established as a species of *Brevibacterium* (Collins et al., 1980), and "*C. marismortui*" has been placed in the new genus *Chromohalobacter* (Ventosa et al., 1989). The production of lipases by "*Chromobacterium viscosum*" has been reported. This bacterium does not belong in *Chromobacterium*; it is a Gram-positive bacterium whose exact taxonomic affiliation is unknown and which has been cited as *Corynebacterium* sp., *Micrococcus* sp., or *Arthrobacter* sp., (De Ley et al., 1978). Two strains isolated from *Psychrotia nairobensis* and *Ardisia crispa* and formerly named "*C. lividum*" or "*C. folium*" have been reclassified as *Sphingomonas yanoikuyae* (Takeuchi and Hatano, 1998). "*Chromobacterium chocolatum*" has been reclassified as *Microbacterium chocolatum* (Takeuchi et al., 1995), and "*C. typhiflavum*" strains (Pedersen et al., 1970) have been reclassified as *Chryseomonas luteola* and *Flavimonas oryzihabitans* (Holmes et al., 1987).

The genus *Chromobacterium* is of interest because it produces substances of importance to agriculture, medicine, and industry. Several of these have been reported in Gillis and De Ley (1992). *Chromobacterium* spp. have been reported to produce chitinase and to reduce the hatch of the nematode *Globodera rostochiensis* (Cronin et al., 1997); the production of a set of chitinolytic enzymes is regulated by an endogenous AHL (Chernin et al., 1998).

Depsipeptide, a cyclic peptide produced by *Chromobacterium violaceum* WB968, has potent antitumor activity against human tumor cells (Ueda et al., 1994; Chan et al., 1997). *Chromobacterium violaceum* strain DSMZ 30191 accumulate a homopolyester of 3-hydroxyvaleric acid up to high percentages of the cellular dry matter when grown on valeric acid (Steinbüchel et al., 1993; Marchessault et al., 1995; Steinbüchel and Schmack, 1995). Several enzymes and metabolites from *Chromobacterium* members have been intensively studied, including phenylalanine hydroxylase (Carr and Benkovic, 1993; Carr et al., 1995), L-tryptophan 2′,3′-oxidase (Hoshino et al., 1993b; Genet et al., 1995; Hammadi et al., 1997), indoloxygenase (Cheah et al., 1998), cytosine deaminase (Kim and Yu, 1998), and serine hydroxylmethyltransferase (Shirazi-Beechey and Knowles, 1984).

List of species of the genus Chromobacterium

1. **Chromobacterium violaceum** Bergonzini 1881, 153[AL]
   vi.o.la′ce.um. L. adj. *violaceum* violet colored.

   The description is the same as that given for the genus. Other characters are as shown in Table BXII.β.86.

The mol% G + C of the DNA is: 65–68 (T_m).
Type strain: MK, ATCC 12472, NCIMB 9131, NCTC 9757.
GenBank accession number (16S rRNA): M22510.

TABLE BXII.β.86. Characteristics of *Chromobacterium violaceum*[a]

Characteristics	*Chromobacterium violaceum*
Colonies on 1/4 nutrient agar:	
Spreading	−
Gelatinous	−
Pigmented	[+] (87)
Zoning of pigment	[−] (25)
Growth on:	
Minimal medium	+
4X Strength nutrient agar	+
Growth at:	
4°C	−
30°C	+
37°C	+
Growth in:	
1% NaCl	+
2% NaCl	+
4% NaCl	−
Growth at:	
pH 4	+
pH 3	−
Formation of cell chains	[−] (12)
Formation of filaments	[−] (15)
Acid from:	
L-Arabinose	−
D-Cellobiose	−
D-Fructose	+
D-Galactose	−
Gluconate	+
D-Glucose	+
Glycerol	+
Glycogen	+
myo-Inositol	−
Inulin	[−] (12)
Lactose	−
D-Maltose	−
D-Mannitol	−
D-Mannose	+
Melezitose	−
N-acetylglucosamine	+
D-Raffinose	−
D-Sorbitol	−
Starch	[+] (87)
Sucrose	[−] (25)
Trehalose	+
D-Xylose	−

(*continued*)

TABLE BXII.β.86. (*cont.*)

Characteristics	*Chromobacterium violaceum*
Catalase	+
Oxidase	+
Nitrate reduction	[+] (87)
Nitrite reduction	[−] (25)
Production of HCN	+
Egg yolk reaction	+
Hemolysis	+
Carbon sources:	
Acetate	[+] (87)
Citrate	+
Fumarate	+
Glycerate	−
Lactate	+
Malate	+
Propionate	[+] (87)
Pyruvate	+
Succinate	+
Tartrate	−
Hydrolysis of:	
Arginine	+
Casein	+
Esculin	−
Gelatin	+
Starch	−
Antimicrobial agents (per disk):	
Ampicillin, 25 μg	R
Cephaloridine, 25 μg	R
Colistin sulfate, 10 μg	R
Chloramphenicol, 10 μg	s
Chlortetracycline, 10 μg	S
Furazolidone, 50 μg	S
Kanamycin, 30 μg	S
Neomycin, 10 μg	s
Nalidixic acid, 30 μg	S
Nitrofurantoin, 200 μg	S
Oxytetracycline, 10 μg	S
Penicillin G, 1.5 U	R
Streptomycin, 10 μg	s
Sulfafurazole, 100 μg	R
Sulfafurazole, 500 μg	s
Vibriostatic agent O/129, 50 μg	R

[a]Symbols: +, all strains positive; [+], positive for 80% or more strains; d, positive for 31–79% strains; [−], positive for 30% or fewer strains; −, negative for all strains; R, resistant; S, sensitive; s, slightly sensitive. Numbers in parentheses indicate the % of strains giving positive reactions.

Genus V. *Eikenella* Jackson and Goodman 1972, 74[AL]

EDWARD J. BOTTONE, FRANCIS L. JACKSON AND YVONNE E. GOODMAN

Ei.ke.nel' la. M.L. dim. ending -*ella*; M.L. fem. n. *Eikenella* named after M. Eiken, who first named the type
species of the genus.

Straight rods, 0.3–0.4 × 1.5–04.0 μm, unbranched, with rounded ends and a regular morphology. Short filaments are occasionally formed. Nonsporeforming. Gram negative. **Nonmotile**, possessing no flagella; however, a "twitching motility" may occur on agar surfaces. **Facultatively anaerobic.** Optimal growth temperature, 35–37°C. **Colonies may appear to corrode the surface of the agar**; noncorroding strains may also occur. Nonhemolytic; a slight greening of blood media around colonies may occur. **Oxidase positive** (Kovacs method). **Negative for catalase, urease, arginine dihydrolase, and indole. Lysine-decarboxylase positive.** Nitrates are reduced to nitrites. **No acid is formed from glucose or other carbohydrates. Hemin is usually required for growth under aerobic conditions.** Occur in the human mouth and intestine; can be opportunistic pathogens.

The mol% G + C of the DNA is: 56–58.

Type species: **Eikenella corrodens** (Eiken 1958) Jackson and Goodman 1972, 75 (*Bacteroides corrodens* Eiken 1958, 415.)

FURTHER DESCRIPTIVE INFORMATION

Electron micrographs of *E. corrodens*, the only species, negatively stained with phosphotungstate show a finely convoluted (cerebral) cell surface. Sections stained with ruthenium red-OsO$_4$ show a cytoplasmic membrane and outer membrane characteristic of Gram-negative bacteria. A slime layer, loosely organized and fibrous, was present on strains examined by Progulske and Holt (1980). The slime layer is associated with the outer surface of the outer membrane, and it is possible that the pilus-like structures demonstrable by negative staining represent components of this layer modified by preparation techniques (Jackson et al., 1971; Progulske and Holt, 1980). Scanning electron microscopy of 7-d-old cultures shows fibrillar material connecting cells (Progulske and Holt, 1980). Adherence of *E. corrodens* to cell surfaces of host tissues is mediated by a cell-associated *N*-acetyl-D-galactosamine-specific, lectin-like substance (Ebisu and Okada, 1983). The lectin is also involved in coaggregation with other bacteria, forming dental plaque and causing proliferation of B-cells in peridontal lesions (Ebisu et al., 1988). The gene encoding a component protein of the lectin-like adhesin complex has been cloned (Yumoto et al., 1996).

Lipopolysaccharide from the organism has been reported to have endotoxin activity, whereas the slime layer has little endotoxin activity, but may be immunosuppressive (Behling et al., 1979). A component with endotoxin activity and containing 0.5% ketodeoxyoctonate, probably a lipopolysaccharide, has been obtained from cells by phenol–water extraction followed by differential centrifugation and gel filtration. It may represent a group antigen, and is distinct from type-specific outer membrane protein antigens, which may also be present (Maliszewski et al., 1983). This major outer membrane protein has a molecular weight of 42,000 Da. *E. corrodens* lipopolysaccharide also demonstrates mitogenic activity different from that elicited by conventional lipopolysaccharide (Progulske et al., 1984).

The organisms appear to be nonmotile according to conventional tests. Corroding colonies (see below) may show spreading edges, and microscopic observation of corroding strains growing on agar surfaces has shown that a form of surface translocation termed "twitching motility" occurs (Henrichsen, 1975a; Henrichsen and Blom, 1975; Schröter, 1975). This motility is correlated with the presence of asymmetrically arranged pili, each with a molecular mass of about 14.8 kDa and an N-terminal amino acid sequence, suggestive of type IV pilins (Hood and Hirschberg, 1995). "Twitching motility" involves small, intermittent jerks leading to displacement over only short distances, not regularly related to the long axis of the cell, at speeds of 1–2 to 2–5 mm/min. The use of cover slips over growth on agar surfaces may prevent this movement, as organisms adhere to the glass, and the phenomenon is dependent on the presence of a thin film of water at the surface of the medium, as is found in cultures incubated in a humidified atmosphere.

Two types of colonies may occur on agar media. Typical "corroding" strains are so named because the colonies appear to corrode the surface of the agar. The organisms penetrate into the surface of the medium and, under humid conditions, tend to spread by twitching motility. The colonies appear as if they are in small depressions in the agar surface, probably because of a combination of spreading surface growth, localized physical alterations of the medium, and optical properties of the colony. The appearance of pitting is not produced on inspissated serum medium. Problems of interpretation of these features have been discussed by Jackson et al. (1971) and by Khairat (1967). Colonies of corroding strains growing on Columbia or trypticase soy agar with 5% sheep's blood at 36°C under 10% CO$_2$ are dry, flat, and radially-spreading, with irregular peripheries (Bottone et al., 1973). With more prolonged incubation, colonies display a characteristic morphology consisting of a clear, moist, glistening center devoid of growth, encircled by a highly refractile, speckled, pearl-like zone of growth that is in turn surrounded by an outer nonrefractile perimeter of spreading growth (Fig. BXII.β.69). Colonies are small (0.2–0.5 mm at 24 h; 0.5–1.0 mm at 48 h). The spreading edge may give colonies a final diameter of ~3.0 mm (Jackson et al., 1971). Colony sizes are similar on cystine–hemin agar. Noncorroding strains, forming colonies 0.5–1.0 mm in diameter that are translucent and dome-shaped, may be isolated by selection from corroding strains and may also be encountered upon primary isolation. These variants do not produce colonies with spreading edges, do not exhibit twitching motility and lack pilus-like surface appendages (Henrichsen, 1975a).

Plate cultures have an odor described as "bleach-like" or as resembling that of *Haemophilus* and *Pasteurella* species.

Growth in fluid media is usually described as poor, but may be improved by addition of cholesterol (10 g/ml; Henriksen, 1969b) or 3% blood serum. The addition of 0.2% agar improves growth (Jackson et al., 1971). In thioglycollate broth, granular growth develops after 3 days in a band 1 cm below the surface. In glucose broth, growth may produce uniform turbidity or small granules adherent to the sides of the tube (Bottone et al., 1973). Granule formation may be related to the presence of pili.

The optimal temperature for growth is 35–37°C. At 25°C, minute colonies are visible in 5–7 days. Growth is good at 40°C but poor at 42°C. No growth occurs at 44°C. The optimal pH for growth is 7.3.

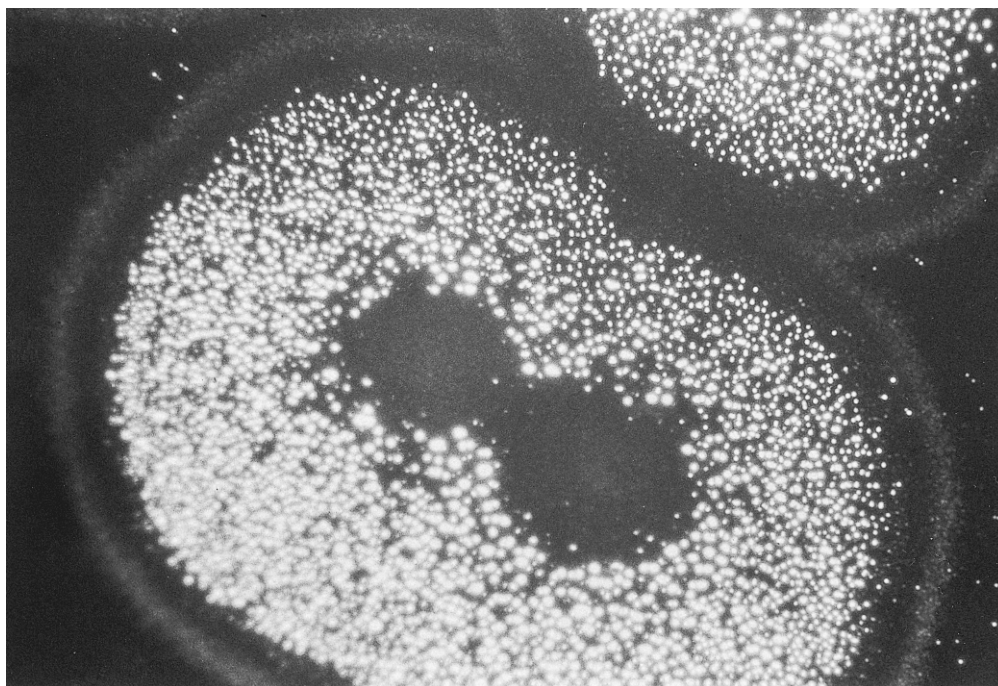Hemin (5–25 g/ml) is required for aerobic growth of freshly

FIGURE BXII.β.69. Colony of *E. corrodens* viewed by oblique lighting, showing a clear, moist center circled by a highly refractive, speckled, pearl-like zone that is surrounded by a perimeter of spreading growth. (Reproduced with permission from E.J. Bottone et al., *American Journal of Clinical Pathology 59:* 560–566, 1973 ©American Society of Clinical Pathologists.)

isolated strains, but not for anaerobic growth. The hemin requirement may be influenced by other constituents of the medium, as indicated by the demonstration that addition of cystine (0.005%) raises the minimum hemin requirement to 20–25 g/ml. Under these conditions, the colony size is increased. Cystine at 0.1% is strongly inhibitory to growth at all hemin concentrations that support growth in the absence of added cystine (Jackson et al., 1971).

Under aerobic or anaerobic conditions, growth of freshly isolated strains is enhanced by 5–10% CO_2. Repeated subculture in air leads to a loss of this response (Hill et al., 1970), but it is retained if strains are repeatedly transferred in a CO_2-enriched atmosphere (Jackson et al., 1971). Strains that have been tested for bile tolerance are inhibited under aerobic conditions by 5 or 10% bile; under anaerobic conditions, growth has been reported to occur in the presence of 10% bile, and there is evidence that this resistance may be higher under microaerobic conditions in the presence of nitrate (Jackson et al., 1971).

Organisms grown anaerobically or aerobically, with or without added CO_2, are strongly oxidase-positive by the method of Kovács (1956), using either dimethyl-*p*-phenylenediamine or the tetramethyl reagent. The standardized tube test (Jackson and Goodman, 1972) can also be used, and it shows that the reaction is azide-sensitive.

Spectroscopic examination has shown the presence of cytochromes of the *b* and *c* groups. Ubiquinone, but not demethylmenaquinone, is present (Holländer and Mannheim, 1975). Growth yields of oxygen-limited cultures are not increased by fumarate. Energy metabolism in *E. corrodens* proceeds via oxidative deamination of key amino acids (proline, glutamate, serine, and glutamine) linked to a respiratory chain, with nitrate,

the ultimate electron acceptor, reduced to nitrite in the process (Gully and Rogers, 1996).

An examination of six strains of *E. corrodens* by gas–liquid chromatography/mass spectrophotometry has shown that the organisms contain hexadecanoic and octadecenoic acids as major fatty acid constituents (Prefontaine and Jackson, 1972). This is in contrast to *Bacteroides ureolyticus*, which has a high content of octadecenoic acid but a low content of hexadecanoic acid.

Antigenic differences between strains have been demonstrated by agglutination reaction and also by immunodiffusion studies, in which up to four antigens have been detected (although some strains lack one or two components) (Jackson et al., 1971). Badger and Tanner (1981) have divided 46 strains into four groups by microagglutination tests. Maliszewski and Badger (1980) have isolated a group antigen common to three serotypes. It appears to be a lipopolysaccharide containing 0.5% ketodeoxyoctonate and showing endotoxin activity by the Limulus amoebocyte lysate test. A type-specific antigen (protein, sensitive to trypsin and heat) has been prepared from one strain. According to Johnson et al. (1978), endotoxin prepared from a corroding strain of *E. corrodens*, in contrast to typical endotoxins, which tend to increase microviscosity of cell membranes, has the unusual effect of causing a decrease in microviscosity of cell membranes, a finding these authors attributed to unique characteristics of the *E. corrodens* endotoxin. Indeed, Progulske et al. (1984) have shown that *E. corrodens* endotoxin is nonclassical, as it contains little 2-keto-3-deoxyoctulosonic acid and heptose and no detectable 9-hydroxy fatty acids.

Some anaerobic Gram-negative rods may show a degree of cross-antigenicity with *E. corrodens* (Robinson and James, 1973), but as pointed out by Jackson and Goodman (1978) the differ-

ence in mol% G + C content of the DNA between *E. corrodens* and these anaerobes precludes a close genetic relationship, and the designation by Robinson and James of strain NCL-20 (now known to be a strain of *Bacteroides ureolyticus*) as a "link" strain between the facultative and anaerobic species is not justifiable.

For determination of antibiotic susceptibility, best results are obtained by standardized-inoculum plate-dilution methods, but other techniques have been used with variable results (Hill et al., 1970; Jackson et al., 1971; Zinner et al., 1973; Brooks et al., 1974; Robinson and James, 1974; Labbé et al., 1977; Goldstein et al., 1978; Slee and Tanzer, 1978). The reports presently available indicate that most strains are susceptible to penicillin G, ampicillin, and cefoxitin, but resistant to penicillinase-resistant penicillins and moderately susceptible or resistant to cephalothin, cephapirin, and cephaloridine. Resistance to aminoglycosides is variable, but usually sufficient to preclude clinical effectiveness. Strains are often susceptible to chloramphenicol, tetracycline, rifampicin, and colistin. Resistance to lincomycin, clindamycin and metronidazole is a constant feature. Two β-lactamase-producing strains of *E. corrodens* have been isolated from an intra-abdominal abscess and an abdominal wound abscess of a 10-year-old and a 2-year-old boy, respectively, hospitalized in San Sebastian, Spain (Trallero et al., 1986).

There is good evidence that *E. corrodens* alone may be pathogenic in humans. When isolated from lesions, it is usually present in mixed culture with other facultative bacteria or with anaerobic bacteria; however, in 10–15% of positive specimens, it is present in pure culture, and it may cause serious diseases. In some cases, it may be the sole survivor in antibiotic-treated mixed infections. Holm (1950) has noted the presence of "corroding bacilli" in actinomycotic lesions, and Reinhold (1966) has studied strains from human sources. Marsden and Hyde (1971) and Kaplan et al. (1973) have reported infections in children. Khairat (1967) has recovered corroding bacilli from 16% of blood cultures drawn 1 min after dental extraction, but the true identity of some of the strains remains uncertain. King (1964) included *E. corrodens* (termed "HB-I") in a discussion of unusual pathogenic Gram-negative bacteria. Brooks et al. (1974) have produced abscesses by injecting mixtures of *E. corrodens* and streptococci, with further potentiation by the addition of methylphenidate, thus simulating lesions found in drug addicts. Experimental endocarditis, rarely fatal and seldom bacteremic, has been produced in catheterized rabbits (Badger et al., 1979); the organism can be shown in vegetation by fluorescent-antibody staining. *E. corrodens* has been associated with endocarditis, osteomyelitis, and septicemia in intravenous drug abusers, and may be related to the practice of "licking needles or skin" prior to injection (Angus et al., 1994; Olopoenia et al., 1994; Swisher et al., 1994).

The possible role of *E. corrodens* in the production of periodontal disease with bone destruction has been discussed and investigated (Socransky, 1977; Johnson et al., 1978; Listgarten et al., 1978; Behling et al., 1979; Progulske and Holt, 1980). *E. corrodens* is frequently found in subgingival plaque in patients with advanced periodontitis (Müller et al., 1997b). Coaggregation with other bacterial species (*Capnocytophaga*, *Actinobacillus*, streptococci) in dental plaque and the ability to elaborate a toxin in gingival sulcus (Levine and Miller, 1996) that may damage surrounding cells both contribute to periodontal disease. Other potential virulence factors operative in periodontal disease include a thiol-dependent hemolysin and other hydrolytic enzymes (Allaker et al., 1994). Infection of the mouth, upper and lower respiratory tract, sinuses, lips, and face is sometimes reported

(Schröter and Stawru, 1970; Jackson et al., 1971; Marsden and Hyde, 1971; Bottone et al., 1973; Carruthers and Sommers, 1973; Kaplan et al., 1973; Brooks et al., 1974; Rubenstein et al., 1976; Goodman, 1977; Piéron and Mafart, 1977; Dudley et al., 1978; DeMello and Leonard, 1979; Jones and Romig, 1979; Colloc et al., 1980; Mégraud et al., 1981; Knudsen and Simko, 1995). Serious infections, including brain abscesses, endocarditis, pneumonia, osteomyelitis, and septic arthritis (Johnson and Pankey, 1976), have been encountered. Transfer from the mouth through human bites and "clenched fist" injuries has resulted in more than 60 cases (Bilos et al., 1978; Goldstein et al., 1978).

The ability of *E. corrodens* to survive in the intestine leads to its presence in abdominal infections, including wound infection, abscesses, and peritonitis of the liver and other viscera (Quinlivan et al., 1996).

E. corrodens is regarded by several investigators as an opportunistic pathogen, particularly likely to produce infection in compromised hosts. Infection is often polymicrobic and is particularly associated with a *Streptococcus* species (Jackson et al., 1971; Bottone et al., 1973; Dorff et al., 1974; Shinhar et al., 1980; Flesher and Bottone, 1989; Quinlivan et al., 1996). Young et al. (1996) have shown that both coaggregation and growth stimulation occur between *E. corrodens* and viridans streptococci of the "*Streptococcus milleri*" group, which may enhance the establishment of mixed infections with these species.

ENRICHMENT AND ISOLATION PROCEDURES

Specimens should be plated on blood agar medium (5% sheep or horse blood, Columbia, or Oxoid No. 2 base, or similar media) and incubated anaerobically and aerobically (in each case with 5–15% CO_2) at 35–37°C. About half of the strains isolated have been recovered from the anaerobic plates but will grow aerobically on first subculture. This may be partly accounted for by suppression of associated organisms and by faster initial growth anaerobically upon transfer from relatively anaerobic body sites to artificial media. In addition, the typical corroding appearance of colonies may develop better under the humid conditions that are usual in anaerobic cultures, leading to easier recognition of the organism. For further information on media and atmospheres of incubation, see Goldstein et al. (1981).

Isolation from mixed infections is facilitated by the use of the medium described by Slee and Tanzer (1978), which contains clindamycin (5 μg/ml), KNO_3 (2.0 mg/ml), and hemin (5.0 g/ml) in agar-solidified Todd–Hewitt medium.

Aerobic plates should be maintained in a humid atmosphere during incubation to encourage production of typical colonies, e.g., in properly humidified CO_2 incubators, or in plastic bags gassed with a CO_2/air mixture and containing wet absorbent paper. Plate cultures should be examined daily for up to 5 days.

MAINTENANCE PROCEDURES

Cultures on blood agar plates should be subcultured at weekly intervals to avoid loss of viability. They may be stored in plastic bags at 4°C after 3 d of incubation.

For preservation in liquid nitrogen, a portion of the growth from a plate is transferred to 1 ml of horse serum, placed in a sterile screw-capped plastic vial and stored in a liquid-nitrogen freezer. Survival for at least 2 years has been reported (Labbé et al., 1977).

For freeze-drying, growth from a single plate is washed off with a suspending medium (either bovine serum containing 7.5% glucose or double-strength reconstituted dried skim milk),

placed in 0.2-ml volumes in freeze-drying tubes, and lyophilized. Freeze-dried cultures in vacuum-sealed glass ampules should be stored in a refrigerator (4°C). Some strains may be serum-sensitive, and, for these, the milk medium may be preferable. The milk should be free from antibiotics. Samples of cultures preserved by any method should be tested for viability before storage of batches.

DIFFERENTIATION OF THE GENUS *EIKENELLA* FROM OTHER GENERA

Differentiation of *Eikenella* from morphologically similar microorganisms (Chadwick et al., 1995), especially *H. paraphrophilus*, is shown in Table BXII.β.87. Additional features that distinguish *E. corrodens* from *Bacteroides ureolyticus* are presented in Table BXII.β.88.

TAXONOMIC COMMENTS

The genus has been defined to include facultative organisms formerly grouped under the name *"Bacteroides corrodens"* (Eiken, 1958). Examination of strains to which this name had been applied revealed that they are heterogeneous. Most are not strict anaerobes, but rather grow in the presence of oxygen on hemin-containing media (Henriksen, 1969a; Hill et al., 1970; Jackson et al., 1971). Certain less oxygen-tolerant strains that are urease-positive have been assigned to the species *Bacteroides ureolyticus* (Jackson and Goodman, 1978). It has been found that some other anaerobic "corroding bacteria" are poorly characterized flagellated organisms (Henrichsen, 1975a; Smibert and Holdeman, 1976; Jackson and Goodman, 1978; Tanner et al., 1981). The statement made by Smibert and Holdeman (1976) that the anaerobic, urease-positive strain VPI 7814 is flagellated is incorrect, as is the report by Brooks et al. (1974) that this strain has a mol% G + C DNA content of 55.0. The true value is 28.4%, and this strain is *B. ureolyticus* and not an atypical *Eikenella* (Jackson and Goodman, 1978).

The organisms termed HB-1 by King (1964) are the same as *E. corrodens* (Jackson and Goodman, 1972; Riley et al., 1973). Similar organisms were included among those referred to as "corroding bacilli" by Holm (1950). The Gram-negative anaerobes forming spreading colonies described by Henriksen (1948) were heterogeneous.

Coykendall and Kaczmarek (1980) have found that 22 *E. corrodens* strains examined have an overall mean mol% G + C DNA content of 56.3, and show at least 70% DNA–DNA relatedness. Variations in relatedness values (70–100%) do not correlate with antigenic differences. It has been concluded that *E. corrodens* can be regarded as a "molecularly homogeneous species," taking into account DNA relatedness, reported mol% G + C ratios, and fatty acid profiles.

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TABLE BXII.β.87. Differentiation of *Eikenella* from similar Gram-negative bacilli that may also produce "corroding" colonies[a]

Characteristic	*Eikenella corrodens*	*Cardiobacterium hominis*	*Actinobacillus actinomycetemcomitans*	*Haemophilus aphrophilus*	*Haemophilus paraphrophilus*	*Kingella kingae*	*Kingella denitrificans*	*Moraxella atlantae*	*Pasteurella multocida*	*Bacteroides ureolyticus*
Oxidase[b]	+	+	+	V	V	+	+	+	W[c]	W
Catalase	–	–	[+][d]	–	–	–	–	+	+	–
Nitrate reduction	+	–	+	+	+	–	+	–	+	+
Nitrite reduction	+	–	–	–	–	–	V	–	–	–
Indole	–	+	–	–	–	–	–	–	+	–
Urease	–	–	–	–	–	–	–	–	–	+
Acid from glucose	–	+	+	+	+	+	+	–	+	–
Lysine decarboxylase	V	–	–	–	–	–	–	–	–	–
Ornithine decarboxylase	+	–	–	–	–	–	–	–	+	–
Gelatin hydrolysis	+	–	–	–	–	–	–	–	–	–
Acid from lactose	–	–	–	+	+	–	–	–	–	–
Anaerobic growth only	–	–	–	–	–	–	–	–	–	+
V-factor dependent	–	–	–	–	+	–	–	–	–	–

[a]Adapted from Weyant et al. (1996); and Chadwick et al. (1995).

[b]Using dimethyl-*p*-phenylenediamine.

[c]W, weakly positive.

[d][+], positive delayed.

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TABLE BXII.β.88. Differentiation of *Eikenella corrodens* from *Bacteroides ureolyticus*[a]

Characteristics	*E. corrodens*	*B. ureolyticus*
Aerobic growth, with hemin	+	−
Urease	−	+
Oxidase test (dimethyl-*p*-phenylenediamine)	+	v
Lysine decarboxylase[b]	+	−
Ornithine decarboxylase[b]	d	−
Gelatin hydrolysis[c]	−	+
Anaerobic growth enhanced by formate–fumarate; succinate is major end product	−	+
Susceptible to clindamycin (5 µg/ml)	−	+
Susceptible to metronidazole (5 µg/ml)	−	+
Anaerobic growth in the presence of 10% bile	+	−
Growth with 0.02% sodium azide	−	+
Odor of plate cultures is "bleach-like"	+	−
Mol% G + C of DNA	56–58	27–29

[a]For symbols see standard definitions.

[b]Møller's method (Møller, 1955).

[c]Frazier (1926) method preferred. 30% trichloroacetic acid may be substituted for mercuric chloride. If conventional 12% gelatin is used, formate–fumarate supplementation is necessary to ensure growth of *B. ureolyticus* (Smibert and Holdeman, 1976). The use of different methods for any test may affect the results. For suitable methods, see Lapage et al. (1968); Hill et al. (1970); Midgley et al. (1970); Jackson et al. (1971); Brooks et al. (1974); and Cowan (1974, appendix C).

List of species of the genus Eikenella

1. **Eikenella corrodens** (Eiken 1958) Jackson and Goodman 1972, 75[AL] ("*Bacteroides corrodens*" Eiken 1958, 415.) *cor.ro′ dens*. M.L. part. adj. *corrodens* gnawing.

   The characteristics are as described for the genus and as listed in Tables BXII.β.87, BXII.β.88, and BXII.β.89. Probably normal inhabitants of the human mouth and intestine. Can be opportunistic.

   The mol% G + C of the DNA is: 56–58 (T_m).

   Type strain: 33/54-55, ATCC 23834, DSM 8340, NCTC 10596.

   GenBank accession number (16S rRNA): M22512.

TABLE BXII.β.89. Other characteristics of *Eikenella corrodens*[a]

Test	Reaction/Result
Anaerobic growth can occur	+
Catalase	−
Oxidase test (di- or tetramethyl-*p*-phenylenediamine)	+
Cytochromes of *b* and *c* groups present	+
Nitrates reduced to nitrites[b]	+
Anaerobic growth enhanced by nitrate	+
Arginine dihydrolase[c]	−
Indole	−
Acid from glucose and other carbohydrates	−
Flagella present	−
Corroding and noncorroding colonies occur	+

[a]For symbols, see standard definitions.

[b]The method of Cook (1950) is convenient for this test (Jackson et al., 1971), but strains giving a negative reaction by this method have been encountered (Hill et al., 1970).

[c]Method of Møller (1955).

Genus VI. **Formivibrio** Tanaka, Nakamura and Mikami 1991c, 580[VP] (Effective publication: Tanaka, Nakamura and Mikami 1991a, 494)

THE EDITORIAL BOARD

For.mi.vib′ ri.o. M.L. n. *acidum formicum* formic acid; M.L. masc. n. *Vibrio* that which vibrates, a generic name; M.L. masc. n. *Formivibrio* the formic acid forming vibrio.

Genus description is the same as for the description of *Formivibrio citricus*.

   Type species: **Formivibrio citricus** Tanaka, Nakamura and Mi-

kami 1991c, 580 (Effective publication: Tanaka, Nakamura and Mikami 1991a, 494.)

List of species of the genus Formivibrio

1. **Formivibrio citricus** Tanaka, Nakamura and Mikami 1991c, 580[VP] (Effective publication: Tanaka, Nakamura and Mikami 1991a, 494.) *ci′ tri.cus*. M.L. adj. *citricus* pertaining to citric acid.

   Gram-negative, curved rods with tapered or rounded

ends, 0.5–0.6 × 1.1–2.5 µm, occurring singly or in pairs. Motile in an active tumbling manner by means of a single polar flagellum. Colonies are colorless, translucent, circular, convex, with an entire margin, up to 0.2 mm in diameter. Anaerobic, having a fermentative type of metabolism. Cat-

alase negative. Do not reduce nitrate, sulfate, sulfite, thiosulfate, or S^0. Do not hydrolyze esculin, urea, or gelatin. Do not produce indole from tryptophan. *S*-Citramalate, citrate, mesaconate, and pyruvate are fermented to acetate and formate and serve as carbon and energy sources. Do not utilize acetoin, acrylate, citraconate, *R*-citramalate, crotonate, fumarate, L-glutamate, glycerol, H_2/CO_2/acetate, lactate, DL-threo-β-methylaspartate, L-malate, maleate, oxalate/acetate, peptones, yeast extract, or carbohydrates.

Cells contain β-hydroxymyristic acid ($C_{14:0\ 3OH}$). Cells wall contains *meso*-diaminopimelic acid. Temperature range 30–35°C; no growth at 25°C or 40°C. Optimal pH 7.6 (range 7.1–7.9); no growth at pH 6.7 or 8.2. Specific growth rate on *S*-citramalate under optimal conditions is 0.12/h. Habitat: anoxic freshwater mud.

The mol% G + C of the DNA is: 59–61 (HPLC).
Type strain: CreCit1, ATCC 49791, DSM 6150.
GenBank accession number (16S rRNA): Y17602.

Genus VII. **Iodobacter** *Logan 1989, 455*[VP]

NIALL A. LOGAN

I.o.do.bac′ ter. Gr. adj. *ioeides* violet-colored; M.L. masc. n. *bacter* the equivalent of Gr. neut. n. *bakterion* a small rod; M.L. masc. n. *Iodobacter* a violet-colored, small rod.

Straight, **round-ended rods, 0.7 × 3.0–3.5 μm, occurring singly, in pairs**, sometimes in chains, and occasionally as long filaments. Definite capsules are not evident. No resting stages known. Gram negative. **Motile by means of both a single polar flagellum and usually one or more subpolar or lateral flagella. Facultative anaerobes.** Grow on ordinary peptone media. **On low-nutrient media, such as nutrient agar containing a quarter of the normal concentration of nutrients, colonies are usually very thin, with rough surfaces and irregular edges, and may spread to 1 cm or more in diameter**; they are of butyrous consistency and are easily emulsified in water. Most strains produce the violet pigment violacein, and this shows most intensely in the centers of spreading colonies, but strains producing nonpigmented colonies (which are easily overlooked) are sometimes encountered. Occasional strains may spread only poorly and may even produce slightly gelatinous colonies, whose appearances are reminiscent of those produced by *Chromobacterium* and *Janthinobacterium* species. On full-strength nutrient agar and other routine, richer media, colonies may show less tendency to spread. Growth in quarter-strength nutrient broth is moderate after 24 h at 25°C, with uniform turbidity and a violet ring at the surface, but no pellicle; strains that produce long filaments may appear to gel in broths. Chemoorganotrophs; **attack carbohydrates fermentatively, producing small amounts of acid, but no gas. Grow at 4–30°C, with optimal growth at ~25°C.**

The mol% G + C of the DNA is: 50–52.

Type species: **Iodobacter fluviatilis** (Moss, Ryall and Logan 1978) Logan 1989, 455 (*Chromobacterium fluviatile* Moss, Ryall and Logan 1981, 216 (Effective publication: Moss, Ryall and Logan 1978, 18.))

FURTHER DESCRIPTIVE INFORMATION

The pigment violacein is produced on or in media containing tryptophan. It is soluble in ethanol but not in water or chloroform, and is readily identified by spectrophotometry and by testing with routine reagents (see section on *Chromobacterium violaceum*).

Colonies of *Iodobacter* on low-nutrient media are characteristically irregular, thin, spreading, and butyrous, with uneven surfaces like beaten copper. The centers show denser growth and thus more intense pigmentation than do the diffuse peripheries (Fig. BXII.β.70). Occasional strains produce slightly gelatinous, non-spreading colonies. Nonpigmented strains are, without doubt, frequently overlooked when isolated. Subcultures of pigmented strains may contain partially or completely unpigmented colonies. An unpigmented strain is most readily recognized by

a colony morphology similar to that of a pigmented strain isolated at the same time under the same conditions. Once it has been confirmed as an oxidase- and catalase-positive, Gram-negative rod, such an isolate should be flagella-stained. If it possesses the characteristic polar and lateral flagella, it should then be subjected to the differential tests for the three genera *Chromobacterium*, *Iodobacter*, and *Janthinobacterium*. Although young cultures of *Aeromonas*, *Pseudomonas*, and *Vibrio* spp. grown on solid media sometimes produce lateral flagella in addition to their usual polar flagella, they give different patterns of results in the differential tests than do the violet-pigmented organisms.

The characteristic flagellar arrangement is best seen in young cultures on solid media. The single polar flagellum is inserted at the tip of the cell, exhibits long, shallow waves and often stains faintly. The lateral flagella are usually long, and 1–4 such flagella, but as many as 8, may occur. They may be inserted subpolarly or laterally, usually exhibit deep, short waves, and stain readily (Fig. BXII.β.71).

Iodobacter fluviatilis strains have been isolated from running fresh water in England and Scotland, and have also been reported from Antarctic lakes and their sediments (Wynn-Williams, 1983).

ENRICHMENT AND ISOLATION PROCEDURES

Iodobacter fluviatilis appears to be a minor member of fresh water floras, and competition with other organisms often results in poor growth and pigmentation and infrequent isolations. For general purposes, the selective medium developed by Ryall and Moss (1975) is recommended: nutrient broth (Oxoid No. 1) at one-quarter strength (¼× NB) is solidified with 1.2% agar (Oxoid No. 3) (to make ¼× Nutrient Agar; ¼× NA) and supplemented, immediately prior to pouring, with filter-sterilized solutions of colistin sulfate, cycloheximide, and sodium deoxycholate (final concentrations, 15, 30, and 300 μg/ml, respectively); the cycloheximide may be omitted if fungal contamination is negligible. Unsupplemented ¼× NA should also be prepared and used both alongside the supplemented medium for isolation and later for subcultures. Spread plates are prepared from undiluted water and from low dilutions and are incubated at 25°C for 5–7 d. Limited trials of the medium described by Keeble and Cross (1977) for isolating *Janthinobacterium* suggest that it is unsuitable for the isolation of *Iodobacter* (Logan, 1989).

MAINTENANCE PROCEDURES

The organisms survive for weeks to months in ¼× NB and for several years in dilute peptone water (0.1% peptone) at 4°C. They
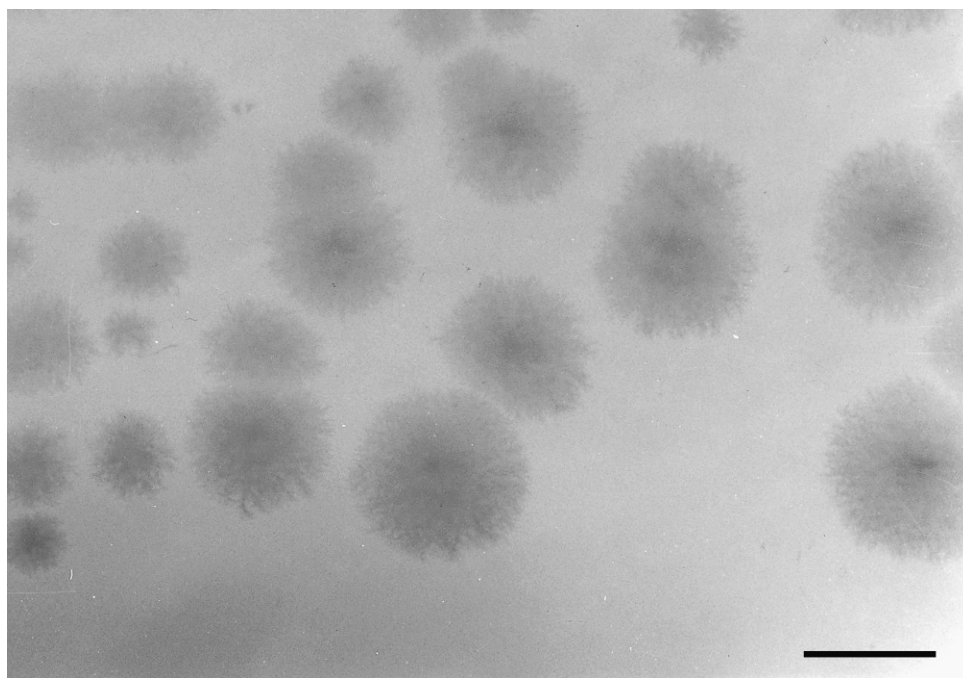
FIGURE BXII.β.70. Colonies of *Iodobacter fluviatilis* ATCC 33051 on quarter strength nutrient agar after 48–72 h at 25°C. Bar = 5 mm.
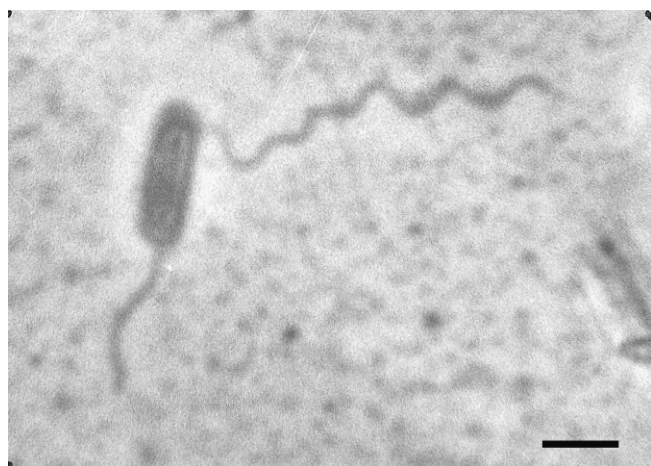

FIGURE BXII.β.71. *Iodobacter fluviatilis* ATCC 33051 stained for flagella by the method of Mayfield and Innis (1977) and showing a long, lateral flagellum with deep waves of short wavelength and a polar flagellum with shallow waves of long wavelength. Bar = 1 μm.

can also be preserved indefinitely by lyophilization or by freezing in nutrient broth containing 15% glycerol.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Methods for demonstrating the production of violacein, for the study of flagellar arrangement and growth temperature, and for demonstrating oxidase reaction, fermentative attack of glucose, anaerobic growth, and acid production from carbohydrates are described in the section on *Chromobacterium violaceum*.

DIFFERENTIATION OF THE GENUS *IODOBACTER* FROM OTHER GENERA

Table BXII.β.90 presents characteristics differentiating the genus *Iodobacter* from the genera *Chromobacterium* and *Janthinobacterium*.

Poorly growing and weakly reacting strains are not unusual (see Logan, 1989, for example), so an identification should be based upon a pattern of reactions rather than on the result of a single test.

TAXONOMIC COMMENTS

Violacein production is a distinctive and important diagnostic feature of *Iodobacter*, but it cannot be emphasized as a taxonomic character, since unpigmented strains are not uncommon (Logan, 1989) and species producing the pigment are found in the genera *Alteromonas*, *Chromobacterium*, and *Janthinobacterium* (Logan and Moss, 1992). Although its mol% G + C range is much lower than that of the other two species in the genus, Moss et al. (1978) allocated their new species of violet chromogen to the genus *Chromobacterium* as *C. fluviatile*, as it is an oxidase- and catalase-positive Gram-negative rod producing violacein and showing the characteristic flagellar arrangement, and because its taxonomic affiliation was unknown. Similarly, *C. violaceum* and *"C. lividum"* were long retained in the same genus on account of these features, even though they show many differences in other respects. In the same year that *C. fluviatile* was described, De Ley et al. (1978) reclassified *"C. lividum"* in the new genus *Janthinobacterium*. Based on nucleic acid hybridization and phenotypic properties and following further nucleic acid hybridization studies (Moss and Bryant, 1982) and numerical analysis of phenotypic characters, *C. fluviatile* was transferred to the new genus *Iodobacter* (Logan, 1989). Phylogenetic relationships inferred from rRNA–DNA hybridization and 16S rRNA sequencing studies support the recognition of these three genera, which represent separate deeply branching lineages of the *Betaproteobacteria*, with *Chromobacterium* and *Iodobacter* being outlying members of the family *Neisseriaceae* (Dewhirst et al., 1989). The adjectival epithet of the only species of the genus, *Iodobacter fluviatile*, has been revised to *Iodobacter fluviatilis* in order to agree in gender with the masculine genus name (Euzéby, 1997).

TABLE BXII.β.90. Patterns of results in tests useful for distinguishing *Iodobacter* from *Chromobacterium* and *Janthinobacterium*[a, b]

Characteristic	*Iodobacter fluviatilis*	*Chromobacterium violaceum*	*Janthinobacterium lividum* (typical)	*Janthinobacterium lividum* (atypical)
Colonies on ¼× NA:				
Spreading	89	0	0	0
Gelatinous	4	0	38	100
Tough	0	0	7	71
Growth at:				
4°C	100	0	97	93
37°C	0	100	0	0
Anaerobic growth	100	100	3	0
D-Glucose:				
Fermented	100	100[c]	0	0[d]
Oxidized	0	0[c]	97	57[d]
Acid from:				
L-Arabinose[e]	0	0	100	100
D-Maltose	100	0	98	93
D-Mannitol[f]	0	0	97	0
Trehalose	100	100	1	100
Lactate utilization	100	0	100	100
Esculin hydrolysis	0	0	100	0
Arginine hydrolysis	0	100	0	0
HCN production	0	100	0	0

[a]Values represent the percentage of strains positive for each characteristic. Number of strains studied: *Iodobacter fluviatilis*, 53; *Chromobacterium violaceum*, 9; *Janthinobacterium lividum* (typical), 68; *Janthinobacterium lividum* (atypical), 14.

[b]From Logan (1989).

[c]Occasional strains are oxidative.

[d]Some strains give no reaction in this test.

[e]Similar patterns of results are obtained with D-cellobiose and D-galactose.

[f]Similar patterns of results are obtained with *myo*-inositol and D-sorbitol.

List of species of the genus Iodobacter

1. **Iodobacter fluviatilis** (Moss, Ryall and Logan 1978) Logan 1989, 455[VP] (*Chromobacterium fluviatile* Moss, Ryall and Logan 1981, 216 (Effective publication: Moss, Ryall and Logan 1978, 18.))

flu.vi.a.ti′lis. L. masc. adj. *fluviatilis* of rivers.

Rods, 0.7 × 3.0–3.5 μm, occurring singly or in short chains, with occasional elongated forms.

Colonies on low-nutrient media are flat, very thin, irregular in outline, spreading and pale violet, with a copper-beaten, slightly rough surface, and they are not gelatinous. A uniform turbidity is produced in nutrient broth with a violet ring at the surface, but usually no pellicle.

Grows on ordinary peptone media. Grows on MacConkey agar, giving violet colonies.

Facultatively anaerobic. Optimal temperature, 25°C; minimum, 4°C; maximum, ~30°C.

Other characteristics are given in Tables BXII.β.90 and BXII.β.91.

Isolated from running fresh water in England and Scotland, and from Antarctic lakes and their sediments.

The mol% G + C of the DNA is: 50–52 (T_m).

Type strain: Moss 165/Sp7, ATCC 33051, DSM 3764, NCIMB 2053, NCTC 11159.

GenBank accession number (16S rRNA): M22511.

TABLE BXII.β.91. Characteristics of *Iodobacter fluviatilis*[a]

Characteristic	*Iodobacter fluviatilis*
Colonies on ¼× NA:	
Spreading	[+] (83)
Gelatinous	[−] (4)
Pigmented	[+] (83)
Zoning of pigment	[−] (6)
Growth on:	
Minimal medium	[+] (96)
4NA	d (45)
Growth at:	
30°C	[+] (83)
37°C	−
Growth in:	
1% NaCl	+
2% NaCl	[−] (9)
4% NaCl	−
Growth at:	
pH 9	[+] (98)
pH 4	[−] (19)
pH 3	−
Formation of cell chains	d (34)
Formation of filaments	[−] (15)
Acid from:	
L-Arabinose	−
Cellobiose	−
Fructose	+
D-Galactose	−
Gluconate	[+] (96)
D-Glucose	+
Glycerol	[−] (13)
Glycogen	−
myo-Inositol	−
Inulin	−
Lactose	−
Maltose	+

(*continued*)

TABLE BXII.β.91. *(cont.)*

Characteristic	*Iodobacter fluviatilis*
D-Mannitol	−
D-Mannose	+
Melezitose	−
N-Acetylglucosamine	+
Raffinose	−
D-Sorbitol	−
Starch	−
Sucrose	−
Trehalose	+
D-Xylose	−
Catalase	+
Oxidase	+
Nitrate reduction	[+] (98)
Nitrite reduction	−
Production of HCN	−
Egg yolk reaction	[−] (13)
Hemolysis	[+] (92)
Carbon sources:	
Acetate	[+] (89)
Citrate	[+] (85)
Fumarate	[+] (89)
Glycerate	d (60)
Lactate	−
Malate	+
Propionate	−
Pyruvate	d (79)
Succinate	[+] (94)

(continued)

TABLE BXII.β.91. *(cont.)*

Characteristic	*Iodobacter fluviatilis*
Tartrate	−
Hydrolysis of:	
Arginine	−
Casein	[+] (90)
Esculin	−
Gelatin	+
Starch	−
Antimicrobial agents (per disk):	
Ampicillin, 25 μg	R
Cephaloridine, 25 μg	R
Colistin sulfate, 10 μg	R
Chloramphenicol, 10 μg	S
Chlortetracycline, 10 μg	S
Furazolidone, 50 μg	S
Kanamycin 30 μg	S
Neomycin 10 μg	s
Nalidixic acid 30 μg	S
Nitrofurantoin, 200 μg	S
Oxytetracycline, 10 μg	S
Penicillin G, 1.5 IU	R
Streptomycin, 10 μg	s
Sulfafurazole, 100 μg	s
Sulfafurazole, 500 μg	s
Vibriostatic agent O/129, 50 μg	R

[a]Symbols: +, all strains positive; [+], positive for 80% or more strains; d, positive for 31–79% strains; [−], positive for 30% or fewer strains; −, negative for all strains; R, resistant; S, sensitive; s, slightly sensitive. Numbers in parentheses indicate the % of strains giving positive reactions.

Genus VIII. **Kingella** *Henriksen and Bøvre 1976, 449^AL emend. Dewhirst, Chen, Paster and Zambon 1993, 497*

ROBBIN S. WEYANT

King.el′la. M.L. *-ella* dim. ending; M.L. fem. n. *Kingella* named after Elizabeth O. King, an American bacteriologist.

Straight rods, 0.6–1.0 × 1.0 to 3.0 μm, with rounded or square ends. Occur in pairs and sometimes in short chains. Endospores are not formed. **Gram negative, but there is a tendency to resist Gram-decolorization.** Nonmotile by normal tests, but may be fimbriated (piliated) and show "twitching motility." **Aerobic or facultatively anaerobic**; grow best aerobically, but can grow weakly under anaerobic conditions on blood agar. Optimal growth temperature, 33–37°C. Two types of colonies occur on blood agar: (a) a spreading, corroding type associated with "twitching motility," fimbriation, and transformation competence, and (b) a smooth, convex type not showing "twitching motility", fimbriation, or competence. **Oxidase positive** (when tested with tetramethyl-*p*-phenylene diamine; the dimethyl reagent may give weak or negative reactions). **Catalase negative.** Coagulated serum is not liquefied. Urease- and indole-negative. Phenylalanine deaminase activity is negative or weak. Chemoorganotrophic. **D-Glucose and a limited number of other carbohydrates are fermented with production of acid, but no gas.** Occur as normal flora in human mucous membranes of the upper respiratory tract. *K. kingae* is an emerging human pathogen, and *K. denitrificans* has been isolated from human infections.

The mol% G + C of the DNA is: 47–58.

Type species: **Kingella kingae** (Henriksen and Bøvre 1968a) Henriksen and Bøvre 1976, 449 (*Moraxella kingae* (Henriksen and Bøvre 1968a) Bøvre, Henriksen and Jonsson 1974, 307; *Moraxella kingii* (sic) Henriksen and Bøvre 1968a, 383.)

FURTHER DESCRIPTIVE INFORMATION

Phylogenetic treatment The genus *Kingella* is in the family *Neisseriaceae*, class *Betaproteobacteria*, and contains three species, *K. kingae*, *K. denitrificans*, and *K. oralis* (Snell, 1984; Dewhirst, et al., 1993).

Cellular morphology and colonial characteristics Cells of *Kingella* strains are characteristically plump, Gram-negative rods or coccobacilli, occurring in pairs or chains. Films from 18-h-old cultures show a tendency to retain the crystal violet of the Gram stain. *Kingella* cells show some pleomorphism, with swollen, irregularly-stained variants. Cells of *Kingella* may show "twitching motility" (Henrichsen et al., 1972; Dewhirst et al., 1993). Twitching motility and competence in genetic transformation are associated with the presence of pili on the cell surface (Frøholm and Bøvre, 1972; Henrichsen, 1972; Weir et al., 1996). *K. kingae* and *K. denitrificans* express type 4 pili that are characteristically long and thin (5–6 nm in diameter) (Weir and Marrs, 1992). Colonies formed by piliated cells are spreading and corroding, in contrast to the smooth, entire, convex colonies formed by nonpiliated cells. Freshly isolated strains of *K. oralis* and *K. denitrificans* produce spreading, corroding colonies (Snell, 1984; Dewhirst et al., 1993). Colonies of *K. kingae* are easily recognized on blood agar in mixed culture by a distinct zone of β-hemolysis surrounding the colonies.

Nutritional and growth requirements All species in the genus are nutritionally fastidious; little or no growth occurs on unsupplemented peptone media. Growth on nutrient agar is only marginally improved by addition of blood or serum, and the colonies remain small, typically 0.5–1.0 mm in diameter after incubation for 48 h. There is no requirement for X or V factors. Although there is no strict requirement for a CO_2-enriched atmosphere, growth of some strains may be enhanced by incubation in 5% CO_2. Growth occurs at 30°C and 37°C but not at 5°C or 45°C. Strains differ in their ability to grow at 22°C. There is some disagreement about of *Kingella* species to grow anaerobically. Henriksen and Bøvre (1976) have described *K. kingae* as aerobic, but Snell and Lapage (1976) have found growth of *K. kingae* and *K. denitrificans* on blood agar in an atmosphere of H_2/CO_2, (95:5), and Dewhirst et al. (1993) have demonstrated growth of *K. oralis* in anaerobic conditions.

Cellular fatty acid composition Cells of *K. kingae* and *K. denitrificans* contain the following fatty acids: *n*-dodecanoic, 3-hydroxydodecanoic, *n*-tetradecanoic, 3-hydroxytetradecanoic, *cis*-9-hexadecenoic, *n*-hexadecanoic, *cis*-9,12-octadecadienoic, *cis*-9-octadecenoic, and *n*-octadecanoic (Weyant, et al. 1996). The cells do not contain *n*-pentadecanoic, 3-hydroxyhexadecanoic, or heptadecenoic acids (Jantzen et al., 1974). Waxes have not been found in *K. kingae* (Bryn et al., 1977).

Antibiotic sensitivity Although *Kingella* strains are normally sensitive to β-lactam antibiotics, sulfonamides, erythromycin, tetracycline, chloramphenicol, ciprofloxacin, and streptomycin, rare cases of antibiotic resistance have been reported. Sordillo et al. (1993) and Minamoto and Sordillo (1992) have reported human infections caused by β-lactamase-positive *K. kingae* and *K. denitrificans* strains. Knapp et al. (1988) have described *K. denitrificans* strains with plasmid-mediated, high-level tetracycline resistance, and Jensen et al. (1994) have identified trimethoprim-resistant *K. kingae* strains. *Kingella* strains are usually resistant to vancomycin.

Pathogenicity *Kingella* species have been associated with infections in humans. Since the 1990s, a significant increase of *K. kingae* septicemia, bone, and joint infections in children under the age of 3 years has been reported (Goutzmanis et al., 1991; Birgisson et al., 1997; Yagupsky and Dagan, 1997; La Scola et al., 1998b; Lundy and Kehl, 1998). *K. kingae* has also been isolated from human corneal ulcer specimens (Mollee et al., 1992). *K. denitrificans* and *K. kingae* have been isolated from blood cultures of individuals with endocarditis (Kerlikowske and Chambers, 1989; Hassan and Hayak, 1993). *K. oralis* has not been associated with invasive infections in humans, although Chen (1996) has reported a significantly higher concentration of this species in dental plaque of individuals with juvenile peridontitis. There have been no published reports of *Kingella* infections in animals or plants.

Natural habitat The natural habitat of *Kingella* is the upper respiratory tract and oral cavity of humans and possibly other primates, where the organisms are present on the mucous membranes as part of the normal flora. Although most *Kingella* isolates have been from human respiratory tract and oral sources, a few isolates of *K. denitrificans* have been obtained from chimpanzee throat and human urogenital specimens (Weyant et al., 1996).

ENRICHMENT AND ISOLATION PROCEDURES

Recovery of *Kingella* from normally sterile clinical specimens, such as blood and synovial fluid, is greatly enhanced by inocu-

lating the specimens into blood culture media (Yagupsky et al., 1992; Yagupsky and Press, 1997; Lejbkowicz et al., 1999). *Kingella denitrificans* has been isolated from specimens with mixed flora by culturing on Thayer–Martin agar (Hollis et al., 1972). *K. kingae* has been selectively isolated on sheep blood agar with 2 µg/ml vancomycin (BAV medium; Yagupsky et al., 1995), and a complex medium containing 5% sheep blood, 5 µg/ml hemin, 0.5 µg/ml menadione, and 1 µg/ml clindamycin has been described by Chen (1996) for the selective recovery of *K. oralis*. Incubation for 48 h may be required for development of colonies of reasonable size on any selective medium. Once isolated, *Kingella* strains may be subcultured on a general, enriched laboratory media, such as blood agar.

MAINTENANCE PROCEDURES

Kingella strains are difficult to maintain by serial transfer, because blood agar cultures become sterile after 6–12 d at room temperature. Preservation is best achieved by freeze drying. Horse serum containing 5% (w/v) *i*-inositol is a suitable suspending medium for freeze drying (Redway and Lapage, 1974). Alternatively, strains may be preserved by suspending fresh cultures in defibrinated rabbit blood and freezing in liquid nitrogen (Weyant et al., 1996).

PROCEDURES FOR TESTING SPECIAL CHARACTERS

The main difficulty in characterizing members of the genus is the poor growth obtained in nonenriched media. Growth is better on solid or semisolid media than in liquid media. Serum promotes a slight, although not a dramatic, improvement of growth, and addition of 5% horse or rabbit serum to test media is worthwhile. For testing the acidification of carbohydrates, rabbit serum is preferable because horse serum contains endogenous maltase activity (Hollis et al., 1983). Due to the slow growth of the organisms, longer than usual incubation periods (3–7 days) may be required to confirm negative results.

Methods suitable for characterization of the genus have been described by Snell et al. (1972), Snell and Lapage (1976), and Weyant et al. (1996).

DIFFERENTIATION OF THE GENUS *KINGELLA* FROM OTHER GENERA

Characteristics by which *Kingella* may be differentiated from phenotypically similar genera and species within the family *Neisseriaceae* are shown in Table BXII.β.92.

TAXONOMIC COMMENTS

These bacteria were historically associated with the genus *Moraxella*. *K. kingae* was originally named *Moraxella kingii* by Henriksen and Bøvre (1968a). The masculine epithet *kingii* was later corrected to the feminine *kingae* by Bøvre et al. (1974). The species was transferred to a newly created genus *Kingella* by Henriksen and Bøvre (1976). In 1972, Hollis et al. described TM-1, a fastidious Gram-negative rod isolated on Thayer–Martin agar from human pharyngeal cultures (Hollis et al., 1972). TM-1 was later classified as *Kingella denitrificans* by Snell and Lapage, who also described *K. indologenes* in the same paper (Snell and LaPage, 1976). In 1990, Dewhirst et al., using 16S rRNA sequence-based methods, found that *K. indologenes* was sufficiently distant from the other *Kingella* species to justify its inclusion in the new genus *Suttonella* as *S. indologenes* (Dewhirst et al., 1990). With the description of *K. oralis* in 1993 (Dewhirst et al., 1993), the genus was again expanded to the 3 currently recognized species.

TABLE BXII.β.92. Differentiation of the genus *Kingella* from phenotypically similar genera and species[a,b]

Characteristics	*Kingella*	*Actinobacillus actinomycetemcomitans*	*Cardiobacterium hominis*	*Eikenella corrodens*	*Haemophilus aphrophilus*	*Moraxella*	*Neisseria*	*Suttonella indologenes*
Cell shape:								
Cocci	–	–	–	–	–	D[c]	D[d]	–
Rods	+	+	+	+	+	D[c]	D[d]	+
Catalase	–	+	–	–	–	+	D[d]	–
Acid from D-glucose	+	+	+	–	+	–	D[d]	+
Acid from D-sorbitol	–	–	+	–	–	–	–	–
Ornithine decarboxylase	–	–	–	+	–	–	–	–
Nitrate reduction	D	+	–	+	+	D	D	–
Indole	–	–	+	–	–	–	–	+

[a]For symbols see standard definitions.

[b]Data from Snell (1984) and Weyant et al. (1996).

[c]*Moraxella catarrhalis* is coccoid. Other *Moraxella* species are bacillary.

[d]*Neisseria elongata* is bacillary. *N. elongata* subsp. *elongata* and *N. elongata* subsp. *nitroreducens* are catalase negative. *N. flavescens, N. cinerea, N. elongata* subsp. *elongata,* and *N. elongata* subsp. *nitroreducens* usually do not form acid from D-glucose.

FURTHER READING

Dewhirst, F.E., C.K.C. Chen, B.J. Paster and J.J. Zambon. 1993. Phylogeny of species in the family *Neisseriaceae* isolated from human dental plaque and description of *Kingella orale,* sp. nov. Int. J. Syst. Bacteriol. *43:* 490–499.

Henriksen, S.D. and K. Bøvre. 1976. Transfer of *Moraxella kingae* Henriksen and Bøvre to the genus *Kingella* gen. nov. in the family *Neisseriaceae.* Int. J. Syst. Bacteriol. *26:* 447–450.

Yagupsky, P. and R. Dagan. 1997. *Kingella kingae*: an emerging cause of invasive infections in young children. Clin. Infect. Dis. *24:* 860–866.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *KINGELLA*

Characteristics differentiating the species of *Kingella* are listed in Table BXII.β.93. Other characteristics of the species are listed in Table BXII.β.94.

TABLE BXII.β.93. Characteristics useful in the differentiation of *Kingella* species[a,b]

Characteristic	*K. kingae*	*K. denitrificans*	*K. oralis*
β-hemolytic	+	–	–
Nitrate reduction	–	+	–
Nitrite reduction	–	+	v[c]
Gas produced from nitrite	–	+	–
Phosphatase activity	+	–	+
Casein digestion	+	–	nd

[a]For symbols see standard definitions; nd, not determined.

[b]Data from Snell (1984) and Weyant et al. (1996).

[c]The type strain of *K. oralis* (CCUG 30450[T]) fails to reduce 0.1% nitrite, but reduces 0.01% nitrite with no gas (data from author's laboratory).

List of species of the genus Kingella

1. **Kingella kingae** (Henriksen and Bøvre 1968a) Henriksen and Bøvre 1976, 449[AL] (*Moraxella kingae* (Henriksen and Bøvre 1968a) Bøvre, Henriksen and Jonsson 1974, 307; *Moraxella kingii* (sic) Henriksen and Bøvre 1968a, 383.) king'ae. M.L. gen. n. *kingae* of King, named after Elizabeth O. King, an American bacteriologist.

The characteristics are as described for the genus and as listed in Tables BXII.β.93 and BXII.β.94.

When grown on blood agar, *K. kingae* colonies produce a characteristic zone of β-hemolysis. This characteristic, along with the tendency of this species to resist Gram-stain decolorization and its failure to produce catalase, may cause some confusion between *K. kingae* and β-hemolytic streptococci in the analysis of specimens from the human throat and oral cavity. The oxidase test, which is generally positive for *K. kingae* and negative for *Streptococcus* species, is a simple and effective differential method.

In recent years, there has been an increased recognition of *Kingella kingae* as an etiologic agent of invasive infections of humans, especially children less than 2 years of age. Since 1985, numerous case reviews describing *Kingella kingae* septic arthritis, osteomyelitis, septicemia, or endocarditis in pediatric patients have been reported. These cases were from various geographic locations, including Sweden (9 cases)

TABLE BXII.β.94. Characteristics of the species of the genus *Kingella*[a,b]

Characteristic	*K. kingae*	*K. denitrificans*	*K. oralis*
Catalase test	−	−	−
Oxidase test	+	+	+
Motility (swimming)	−	−	−
Anaerobic growth (blood agar)	w	w	w
Temperature tolerance, growth at:			
5°C	−	−	nd
22°C	d	d	+
30°C	+	+	+
37°C	+	+	+
45°C	−	−	−
Growth in the presence of 6% NaCl	−	−	−
Bile tolerance, growth in:			
10% bile	−	−	nd
40% bile	−	−	nd
Growth stimulation by bile	−	−	nd
Esculin hydrolysis	−	−	−
Growth on MacConkey agar	−	−	−
Liquefaction of gelatin and coagulated serum	−	−	−
Citrate utilization	−	−	−
Growth in mineral medium with β-hydroxybutyrate	−	−	nd
Formation of intracellular poly-β-hydroxybutyrate (nutrient medium)	−	−	nd
Urease activity	−	−	−
Lipase activity: hydrolysis of:			
Tween 20	−	d	nd
Tween 80	−	−	nd
Lecithinase activity	−	−	nd
Arginine dihydrolase	−	−	−
Ornithine decarboxylase	−	−	−
Lysine decarboxylase	−	−	−
Deoxyribonuclease activity	−	d	nd
H$_2$S production (detection by lead acetate strips)	d	−	+
Susceptible to penicillin (1.0 U/ml)	+	+	nd
β-galactosidase activity (ONPG test)	−	−	nd
Starch hydrolysis	−	−	nd
Oxidation/fermentation test	F	F	F
Acid produced from:			
D-Glucose	+	+	+
Sucrose	−	−	−
Maltose	+	−	−
D-Xylose	−	−	−
Lactose	−	−	−
D-Mannitol	−	−	−
Fructose	−	−	nd
Dextrin	−	d	nd
Adonitol	−	−	nd
L-Arabinose	−	−	nd
Cellobiose	−	−	nd
Dulcitol	−	−	nd
Ethanol	−	−	nd
D-Galactose	−	−	nd
Glycerol	−	−	nd
i-Inositol	−	−	nd
D-Mannose	−	−	nd
Raffinose	−	−	nd
L-Rhamnose	−	−	nd
Salicin	−	−	nd
D-Sorbitol	−	−	nd
Trehalose	−	−	nd

[a]For symbols see standard definitions; w, weak growth; nd, not determined.
[b]Data from Snell (1984), Dewhirst et al. (1993), and Weyant et al. (1996).

(Claesson et al., 1985), Iceland (5 cases) (Birgisson et al., 1997), Australia (10 cases) (Goutzmanis et al., 1991), France (5 cases) (La Scola et al., 1998b), Israel (42 cases) (Yagupsky and Dagan, 1997), and the United States (10 cases) (Lundy and Kehl, 1998). In many cases, *K. kingae* infections occur as sequelae to viral infections, with the viral agents presumably compromising mucosal immunity to allow the seeding of the bloodstream with *K. kingae* from the oral cavity (Waghorn and Cheetham, 1997; Amir and Yagupsky, 1998). Lundy and Kehl (1998) have observed that the apparent increase in *K. kingae* infections corresponds with the vaccine-mediated decrease in *Haemophilus influenzae* infections in young children, suggesting that *K. kingae* is replacing *H. influenzae* as a significant etiologic agent in this population.

The mol% G + C of the DNA is: 47.3–47.4 (T_m).
Type strain: ATCC 23330.
GenBank accession number (16S rRNA): M22517.

2. **Kingella denitrificans** Snell and Lapage 1976, 456[AL]
   de.ni.tri′fi.cans. L. prep. *de* away from; L. n. *nitrum* soda; M.L. n. *nitrum* nitrate; M.L. v. *denitrifico* to denitrify; M.L. part. adj. *denitrificans* denitrifying.

   The characteristics are as described for the genus and as listed in Tables BXII.β.93 and BXII.β.94.

   Kingella denitrificans was first described by Hollis et al. (1972), who encountered isolates of this species on Thayer–Martin selective agar while performing a study to determine pharyngeal carrier rates of *Neisseria meningitidis* and *N. lactamica* in healthy adults. The isolates were originally designated "TM-1" in recognition of their ability to grow on Thayer–Martin agar.

   Kingella denitrificans is commonly found as part of the normal flora of the human pharynx and is generally considered to be of low pathogenicity. Of 60 strains studied at the U.S. Centers for Disease Control and Prevention, only 2 have been from sources, i.e., blood and mandibular abscess, suggestive of invasive infection (Weyant et al., 1996). Rare cases of endocarditis (Hassan and Hayek, 1993), and a case of granulomatous disease in an AIDS patient (Minamoto and Sordillo, 1992) have been reported.

   The mol% G + C of the DNA is: 54.1–54.8 (T_m).
   Type strain: ATCC 33394, DSM 10202, NCTC 10995.
   GenBank accession number (16S rRNA): M22516.

3. **Kingella oralis** Dewhirst, Chen, Paster, and Zambon 1993, 498[VP] (*Kingella orale* (sic) Dewhirst, Chen, Paster, and Zambon 1993, 498.)
   o.ra′lis. L. adj. *oralis* oral, pertaining to the mouth.

   The characteristics are as described for the genus and as listed in Tables BXII.β.93 and BXII.β.94.

   Kingella oralis was originally described by Chen et al. (1990a) as *Eikenella corrodens*-like isolates obtained from human oral cavity specimens. In the original 1993 classification paper, this species was named *K. orale*, but the spelling of the species name was changed to "*oralis*" in 1994 (Dewhirst et al., 1993, 1994). *K. oralis* is found in the human oral cavity and produces corroding colonies similar to those of *E. corrodens*. Biochemical tests useful in differentiating *K. oralis* from *E. corrodens* include acidification of D-glucose and ornithine decarboxylase.

   The mol% G + C of the DNA is: 56–58 (T_m).
   Type strain: ATCC 51147, CCUG 30450.
   GenBank accession number (16S rRNA): L06164.

Genus IX. **Microvirgula** *Patureau, Godon, Dabert, Bouchez, Bernet, Delgenes and Moletta 1998, 781*[VP]

DOMINIQUE PATUREAU

Mi.cro.vir′ gu.la. Gr. adj. *micros* small; L. fem. n. *virgula* twig or rod; M.L. n. *Microvirgula* small twig or rod.

Curved rods, occurring singly, in pairs, or in clusters. **Motile.** Gram negative. **Oxidase and catalase positive. Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor; however, anaerobic respiration occurs in the presence of nitrate, nitrite, and nitrous oxide, with the formation of N_2. **Denitrification is not repressed under aerobic conditions.** Chemoorganotrophic. **Growth occurs on a variety of carbon sources including acetate, succinate, propionate, ethanol, methanol, and glycerol, but not on sugars.** Belongs to the *Betaproteobacteria.* Isolated from an up-flow anoxic filter inoculated with activated sludges.

The mol% G + C of the DNA is: 65.

Type species: **Microvirgula aerodenitrificans** Patureau, Godon, Dabert, Bouchez, Bernet, Delgenes and Moletta 1998, 781.

DIFFERENTIATION OF THE GENUS *MICROVIRGULA* FROM OTHER GENERA

Phenotypically, the genus most closely resembles *Comamonas testosteroni, Alcaligenes faecalis,* and *Thauera mechernichensis.* Table BXII.β.95 lists characteristics that differentiate the genus from these species and from the phylogenetically related organisms *Chromobacterium violaceum* and *Vogesella indigofera.*

TAXONOMIC COMMENTS

In this edition of the *Manual,* the genus *Microvirgula* is placed in the class *Betaproteobacteria,* the order *Neisseriales,* and the family *Neisseriaceae.* Based on studies of the 16S rDNA sequence by Patureau et al. (1998), the genus is most closely related to *Vogesella indigofera* (87.3%) and *Chromobacterium violaceum* (87.9%) (Fig. BXII.β.72).

List of species of the genus Microvirgula

1. **Microvirgula aerodenitrificans** Patureau, Godon, Dabert, Bouchez, Bernet, Delgenes and Moletta 1998, 781[VP]

   ae.ro.de.ni.tri′ fi.cans. Gr. n. *aer* air; M.L. v. *denitrificare* to denitrify; M.L. part. *aerodenitrificans* denitrifying with or in air.

   The characteristics are as described for the genus, with the following additional information. Oxidizable carbon and energy sources include methylpyruvate, acetate, formate, β-hydroxybutyrate, propionate, sebacic acid, succinate, L-asparagine, L-aspartic acid, L-glutamic acid, L-leucine, L-proline, methanol, ethanol, and glycerol.

   The following compounds are not utilized: dextrin, glycogen, xylitol, citrate, α-ketoglutarate, α-ketovalerate, malonate, D-alanine, L-phenylalanine, D-serine, L-threonine, D,L-carnitine, inosine, uridine, 2,3-butanediol, and glucose-1-phosphate, D-galacturonic acid, and D-gluconic acid.

   Optimal pH, 7. Optimal temperature, 35°C; range, 15–45°C. Maximum growth rates (μmax) are 0.37 h^{-1} under aerobic conditions, 0.23 h^{-1} under mixed oxic–nitrate conditions, and 0.11 h^{-1} under strict anaerobic conditions.

   The type strain can co-respire oxygen and nitrogen oxides under O_2-saturated conditions with activity and synthesis of the four denitrifying enzymes, regardless of the aeration conditions. A similar behavior is observed with *Paracoccus pantotrophus, Alcaligenes faecalis* strain TUD, and *Thauera mechernichensis.*

   Lipase activity occurs.

   The mol% G + C of the DNA is: 65.

   Type strain: SGLY2, LMG 18919.

   GenBank accession number (16S rRNA): U89333.

TABLE BXII.β.95. Characteristics differentiating the genus *Microvirgula* from other bacteria[a,b]

Characteristic	*Microvirgula*	*Comamonas testosteroni*	*Alcaligenes faecalis*	*Chromobacterium violaceum*	*Vogesella indigofera*	*Thauera mechernichensis*
Nitrate reduced to nitrite	+	+	−	+	+	+
Nitrite reduced to N_2	+	+	+	+	+	+
Capable of aerobic denitrification	+	−	+	nd	nd	+
Colonies are blue or violet	−	−	−	+	+	−
Growth at 45°C	+	−	nd	−	nd	nd
Oxidative metabolism only; cannot ferment	+	+	+	−	+	+
Substrates utilized:						
Methanol	+	nd	nd	nd	nd	−
Ethanol	+	−	nd	nd	+	+
Glucose	−	−	−	+	−	nd
Citrate	−	+	+	+	+	nd
Acetate	+	−	+	d	nd	+

[a]Symbols: see standard definitions; nd, not determined.

[b]Data from Grimes et al. (1997), Patureau et al. (1998), and Scholten et al. (1999).
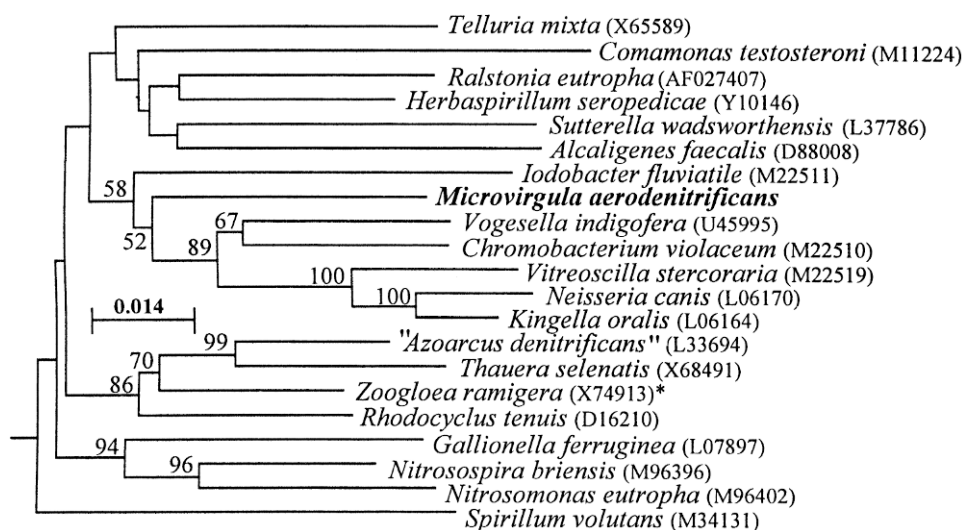
FIGURE BXII.β.72. Phylogenetic tree showing the relationship of *Microvirgula aerodenitrificans* to other members of the class *Betaproteobacteria*. This tree was inferred from 16S rRNA gene sequence data by the neighbor-joining method (Saitou and Nei, 1987). A total of 1120 nucleotide positions were included in the analysis. The tree was rooted with the sequence of *Paracoccus denitrificans*. The scale bar indicates the percentage difference per nucleotide position using the Jukes and Cantor correction (Jukes and Cantor, 1969). Numbers refer to bootstrap values up to 50% for each node out of a total of 500 replicate samplings (Felsenstein, 1985). *, accession number corresponds to strain ATCC 19544. Names are given as cited in the GenBank database. (Reproduced with permission from D. Patureau et al., International Journal of Systematic Bacteriology 48: 775–782, 1998, ©International Union of Microbiological Societies.)

Genus X. **Prolinoborus** Pot, Willems, Gillis and De Ley 1992b, 52^VP

NOEL R. KRIEG

Pro.li.no' bo.rus. L. n. *prolina* the amino acid proline; Gr. adj. *boros* voracious M.L. masc. n. *Prolinoborus* (bacteria) that readily consume proline.

Straight rods, 0.7–0.9 μm in width.. Cells are approximately 5–10 μm in length, but old cultures may contain cells up to 42 μm long. Curved or S-shaped variants have been reported to occur in one strain after prolonged serial transfer. A polar membrane underlies the cytoplasmic membrane. **Intracellular poly-β-hydroxybutyrate granules are present** in the rods. Extensive conversion of the rod-shaped cells to round forms (**"coccoid bodies"**) occurs in older cultures. **Upon initial isolation, the cells form highly viscous flocs, within which the cells swim steadily in straight lines.** The floc-forming ability is gradually lost during subsequent serial transfers, and the growth eventually becomes turbid. Cells have **bipolar flagellar fascicles composed of up to 11 flagella**. The fascicles can be seen clearly by dark-field microscopy and show **unusual and distinctive behavior** when the cells are suspended in ordinary, nonviscous media: helical wave propagation with waves progressing from base to tip, an ability to coil up like springs, and basal bending accompanied by a change in wavelength. **In such nonviscous media the cells do not swim; instead, they exhibit an ineffectual "floundering about" movement;** however, when they are suspended in a medium of high viscosity (10–200 centipoise, obtained by the use of agents such as DNA or methylcellulose "400 centipoise"), they swim steadily in straight lines. Optimal temperature, 30°C; no growth at 20°C or 40°C. **Catalase and oxidase positive. Carbohydrates are not catabolized.** Pyruvate and proline are the most effective sole carbon sources. **Proline can be used a sole source of nitrogen and carbon. Nitrogenase activity** (ability to reduce acetylene) **occurs under microaerobic conditions**. Habitat: pond water.

The mol% G + C of the DNA is: 62–65.

Type species: **Prolinoborus fasciculus** (Strength, Isani, Linn, Williams, Vandermolen, Laughon and Krieg 1976) Pot, Willems, Gillis and De Ley 1992b, 53 (*Aquaspirillum fasciculus* Strength, Isani, Linn, Williams, Vandermolen, Laughon and Krieg 1976, 266.)

FURTHER DESCRIPTIVE INFORMATION

The cell morphology and behavior of the flagellar fascicles is illustrated in Fig. BXII.β.73 and BXII.β.74.

Upon initial isolation, the cells form highly viscous flocs which, when homogenized in a small quantity of water, contain free-swimming cells that move in straight lines within the viscous matrix. The tailing flagellar fascicle is extended behind each cell, while the leading fascicle is either coiled into a polar loop or coiled around the cell. Free cells or cells at the periphery of the flocs are not motile, or their motility is irregular over short distances. After several serial transfers, the floc-forming ability is gradually lost, and the cells no longer swim, although the flagellar fascicles exhibit helical wave propagation, basal bending, and the ability to coil up like springs. The ability to swim only in viscous media may represent an adaptation to the viscous conditions that occur within cell flocs.

The mass conversion of the rod form to the coccoid form is
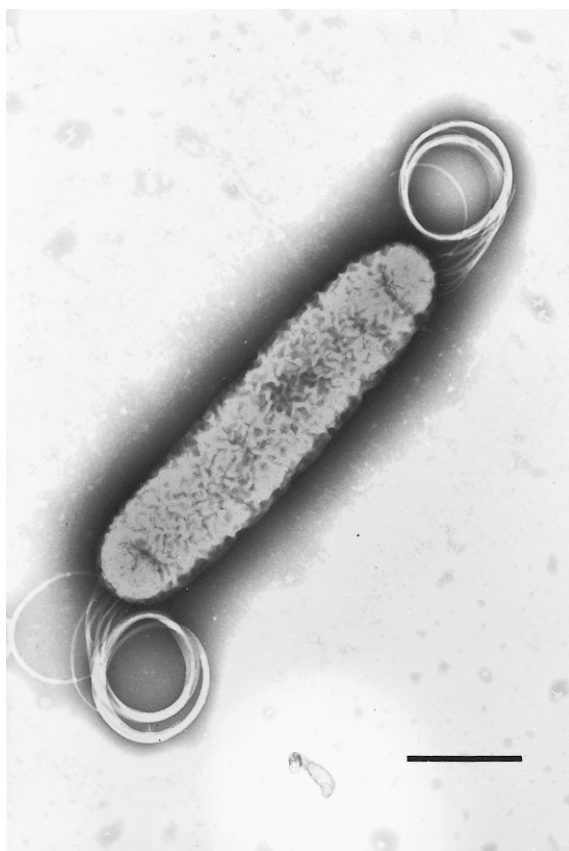
FIGURE BXII.β.73. Formalin-fixed cells of *Prolinoborus fasciculus* showing the bipolar fascicles of flagella coiled into loops. When the fascicles are extended, they have a helical configuration with several waves. Bar = 1.0 μm. (Reproduced with permission from W.J. Strength and N.R. Krieg, Canadian Journal of Microbiology *17:* 1133–1137, 1971, ©National Research Council of Canada.)

FIGURE BXII.β.74. Flagellar behavior in *P. fasciculus. A–C,* flagellar orientation observed in nonviscous media: *A,* flagellar fascicles extended; *B,* coiling into a polar loop; and *C,* basal bending accompanied by a change in wavelength. *D,* orientation of the fascicles in motile cells suspended in a viscous medium; the arrow indicates the direction of swimming.

shown in Fig. BXII.β.75. Koechlein and Krieg (1998) have found that chloramphenicol does not prevent the conversion of the rods to the coccoid form. Attempts to obtain variants that do not convert to the coccoid form have been unsuccessful. Although the coccoid form fluoresces with acridine orange, extensive rRNA degradation has occurred, as indicated by agarose gel electrophoresis. Poly-β-hydroxybutyrate, abundant in the vegetative rods, is not detectable in the coccoid cells. The results suggest that the coccoid form of *P. fasciculus* may be a degenerative form, rather than part of a life cycle.

ENRICHMENT AND ISOLATION PROCEDURES

In studies by Strength et al. (1976), organisms with the characteristic morphological features were found to reach maximal numbers in the surface scum of pond water–hay infusions in 3 d at 30°C. Some enrichment can be achieved by subsequent cultivation of the scum in Pringsheim soil medium[1]. A loopful of

surface pellicle from a 4-d-old enrichment culture is transferred to 10 ml of sterile water. After vigorous shaking to disperse the cell flocs, the suspension is serially diluted in sterile water, and each dilution is used to seed plates of melted, cooled PR medium[2] containing 0.75% agar. After 36 h at 30°C, numerous small (0.1–0.4 mm diameter), white, irregularly shaped colonies (as well as other types of colonies) develop. Each small colony is removed with sterile capillary tubes under a dissecting microscope and the agar plug containing the isolated colony is blown out into a tube containing semisolid (0.15% agar) PR medium. The agar plugs are crushed with a glass rod after the transfer.

After growth occurs, the organisms can be transferred to PFS[3] broth. On initial isolation, the organism forms highly viscous flocs. This floc-forming ability is gradually lost during subsequent transfers, and eventually the strains exhibit homogeneous, turbid growth.

MAINTENANCE PROCEDURES

Strains can be maintained by serial transfer at 48-h intervals in semisolid PFS medium. Incubation beyond 48 h usually results in lack of viability, probably because of extensive formation of the coccoid form. For long-term preservation, centrifuged cells are suspended to high density in PFS broth containing 15% glycerol and stored in liquid nitrogen.

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1. Pringsheim soil medium (Rittenberg and Rittenberg, 1962): place one wheat or barley grain in a large test tube, and cover it with 3–4 cm of garden soil. Fill the tube almost to the top with tap water. Sterilize the medium at 121°C for 30 min.

2. Proline-salts (PR) medium of Strength et al. (1976) (g/l): L-proline, 0.5; K_2HPO_4, 0.45; $MgSO_4 \cdot 7H_2O$, 0.25; $MnSO_4 \cdot H_2O$, 0.001; and $FeCl_3 \cdot 6H_2O$, 0.001. Adjust to pH 7.0 with KOH.

3. Peptone-fumarate-salts (PFS) broth of Strength et al. (1976) (g/l): Bacto peptone (Difco), 10.0; fumaric acid, 2.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; $FeCl_3 \cdot 6H_2O$, 0.002; $MnSO_4 \cdot H_2O$, 0.002; Adjust to pH 7.0 with KOH. The medium can be used in liquid, semisolid (0.15% agar), or solid (1.5% agar) form.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Cultures to be tested for nitrogenase activity should be cultured in nitrogen-free, semisolid malate medium (see the genus *Azospirillum*) supplemented with 0.005% yeast extract. Cultures are incubated for 3 d at 30°C and then sealed with rubber vaccine-bottle stoppers. Acetylene is injected to a final concentration of 10% (v/v), and the cultures are tested for ethylene production by gas chromatography after 1 h of further incubation. Controls using liquid rather than semisolid medium and semisolid medium containing 0.1% $(NH_4)_2SO_4$ should be negative for ethylene production.

DIFFERENTIATION OF THE GENUS *PROLINOBORUS* FROM OTHER GENERA

In most of its physiological properties, *Prolinoborus* is similar to aerobic, heterotrophic, freshwater spirilla such as *Aquaspirillum serpens* (its closest relative). However, the rod shape of the cells, floc formation, and especially the unusual behavior of the flagellar fascicles clearly differentiate *Prolinoborus* from these spirilla. The unusual flagellar behavior, formation of viscous flocs, ability to use proline as a sole carbon and nitrogen source, inability to use carbohydrates, and nitrogenase activity readily differentiate *Prolinoborus* from other aerobic Gram-negative freshwater rods.

TAXONOMIC COMMENTS

Prolinoborus fasciculus was initially classified in the genus *Aquaspirillum* by Strength et al. (1976). However, DNA–rRNA hybridization studies by Pot et al. (1992b) indicated that the genus *Prolinoborus* belongs to rRNA superfamily III (now the class *Betaproteobacteria*), with a difference in $T_{m(e)}$ of more than 6°C with its closest relative, *Aquaspirillum serpens*. Pot et al. regarded this $T_{m(e)}$ difference as support for the classification of *A. fasciculus* into a new genus, *Prolinoborus*. *Prolinoborus fasciculus* is currently the only species in this genus.
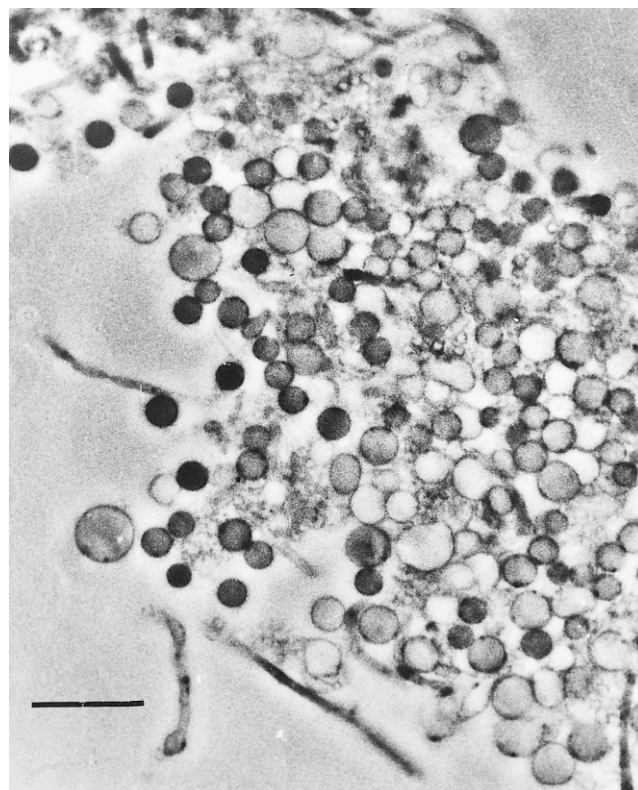
FIGURE BXII.β.75. Coccoid bodies of *P. fasciculus* in peptone–fumarate–salts broth cultures incubated on a shaking machine at 30°C for 48 h. Phase contrast microscopy. Bar = 10 μm.

List of species of the genus Prolinoborus

1. **Prolinoborus fasciculus** (Strength, Isani, Linn, Williams, Vandermolen, Laughon and Krieg 1976) Pot, Willems, Gillis and De Ley 1992b, 53[VP] (*Aquaspirillum fasciculus* Strength, Isani, Linn, Williams, Vandermolen, Laughon and Krieg 1976, 266.)

   fas.ci' cu.lus. L. masc. dim. n. *fasciculus* a small bundle.

   The characteristics are as described for the genus, with the following additional information. No water-soluble brown pigment is formed in the presence of 0.1% tyrosine or tryptophan. Growth occurs in the presence of 1% oxgall. No growth occurs in the presence of 1% glycine and 3% NaCl. No hydrolysis of casein, starch, esculin, or hippurate. Gelatin is hydrolyzed in 4 d at 30°C. Anaerobic growth occurs with nitrate. KNO_3 is reduced only to KNO_2. Phosphatase and urease positive. Indole negative.

   Sole carbon sources, as determined by the method of Strength et al. (1976) include succinate, fumarate, malate, oxaloacetate, pyruvate, lactate, β-hydroxybutyrate, L-alanine, L-glutamate, L-aspartate, L-glutamine, L-asparagine, L-proline, and L-arginine. The following carbon sources are not used: citrate, α-ketoglutarate, malonate, acetate, propionate, butyrate, ethanol, *n*-propanol, *n*-butanol, glycerol, D-fructose, D-glucose, D-xylose, L-arabinose, L-histidine, L-tyrosine, L-phenylalanine, L-hydroxyproline, L-ornithine, L-lysine, L-methionine, L-serine, L-cysteine, L-leucine, L-isoleucine, L-valine, and L-tryptophan.

   The mol% G + C of the DNA is: 62–65 (T_m).
   Type strain: ATCC 27740, LMG 6233.

Genus XI. **Simonsiella** *Schmid in Simons 1922, 504*[AL]

BRIAN P. HEDLUND AND TONE TØNJUM

Si.mon.si.el' la. M.L. dim. *-ella* ending; M.L. fem. n. *Simonsiella* (organism of) Simons; named for H. Simons, who studied the species of this genus.

Organisms that exist in characteristic **multicellular filaments** that are **flat rather than cylindrical** and often segmented into groups of eight cells. The **width of an individual cell is greater than its length. The long axis of an individual cell is perpendicular to the long axis of the filament.** The diameter of the filaments (the width of the individual cells) may vary from about 2.0 to 8.0 μm, and the length of filaments may vary from about 10.0 to over 50.0 μm. Individual cells within the filaments may be from about 0.5 to 1.3 μm long. In thin sections cut perpendicular to the long axis of the filament, the **cells are flattened and curved to yield a crescent-shaped, convex–concave (dorsal–ventral) asymmetry.** The **ends of the individual filaments are rounded.** Gram negative. **Gliding motility** of the entire filament in the direction of the long axis when the flat side of the filament is in contact with a surface. Chemoorganotrophs. Aerobic. Some may produce acid aerobically from carbohydrates. Optimal temperature: 37°C. **Found in the oral cavity of warm-blooded vertebrates.**

The mol% G + C of the DNA is: 41–55.

Type species: **Simonsiella muelleri** Schmid *in* Simons 1922, 504.

FURTHER DESCRIPTIVE INFORMATION

The genus *Simonsiella* is characterized by a unique multicellular morphology. The filaments of *Simonsiella* are distinctive and members of this genus can be recognized by their morphology alone (Figs. BXII.β.76, BXII.β.77, and BXII.β.78). The dorsal-ventral flattening of the filaments is quite striking, as is the fact that the individual cells of the filament are wider than they are long if the long axis of the filament is considered to represent the length of the cells. The cells toward either end of the filament decrease in width, and the terminal cells may be rounded, giving the filaments a tapered appearance with rounded ends. In isolated colonies, some of the filaments may be turned on their sides, which shows the flattening quite clearly. Starr and Skerman (1965) suggested that this type of structure should be called a

trichome—chain of closely apposed bacterial cells—found, for example, in certain genera of the cyanobacteria, the genus *Toxothrix* and the genus *Caryophanon*. From the convex (bottom) side of the filaments there are numerous fine fibrils (Fig. BXII.β.78), which appear to be involved in the adhesion of the filaments to a surface and perhaps in locomotion (Pangborn et al., 1977). In thin sections, the cells appear to have a typical Gram-negative cell wall structure.

Individual filaments glide over the agar surface only when the broad, ventral surface is in contact with the agar. Movement of the filaments over the agar leaves depressed tracks in the agar surface (Fig. BXII.β.77), perhaps indicating a change in the structure of the agar (Pangborn et al., 1977). No flagella have been demonstrated, but the presence of pili (fimbriae) should be assessed. The speed of gliding varies from about 5 to 24 μm/min (Buchanan and Kuhn, 1978). Colonies on BSTSY agar (see Enrichment and Isolation Procedures) may have a pale yellow pigmentation. Most, but not all, strains produce a zone of hemolysis on agar containing horse, sheep, or rabbit blood. No resting stage has been detected.

All isolates of *Simonsiella* are chemoorganotrophic, and the nutrition of the isolates (as well as their classification) parallels their source of origin. The isolates from sheep (*S. crassa*) are generally the most physiologically active, being both proteolytic and saccharolytic (Tables BXII.β.96, BXII.β.97, BXII.β.98, BXII.β.99, and BXII.β.100). In contrast, the isolates from dogs (*S. steedae*) are neither proteolytic nor saccharolytic. Kuhn et al. (1978) have described over 50 isolates of *Simonsiella*. All strains are aerobic, possess cytochrome oxidase, and produce catalase. Good growth occurs between 3° and 40°C, is optimal at 37°C, and does not occur at 45°C. Growth occurs in 1% NaCl but not
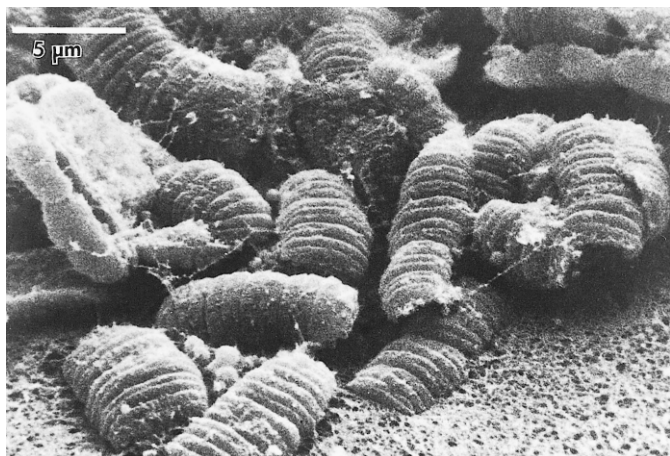

FIGURE BXII.β.76. Scanning electron micrograph of the edge of a colony of *Simonsiella* obtained from a cat. (Micrograph taken by J. Pangborn.) (Reproduced with permission from J. Pangborn et al., Archives of Microbiology *113:* 197–204, 1977, ©Springer-Verlag.)
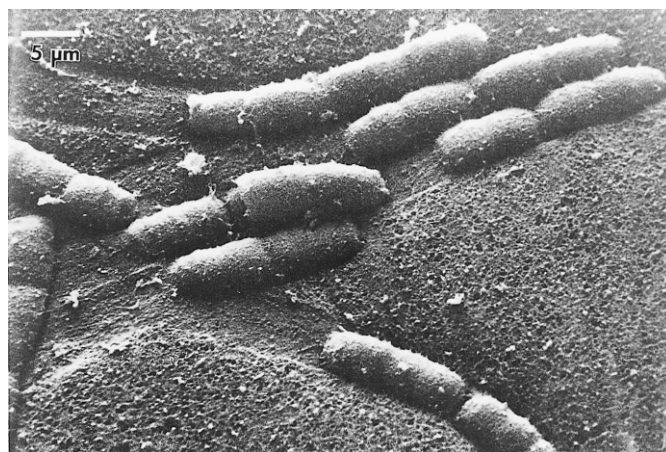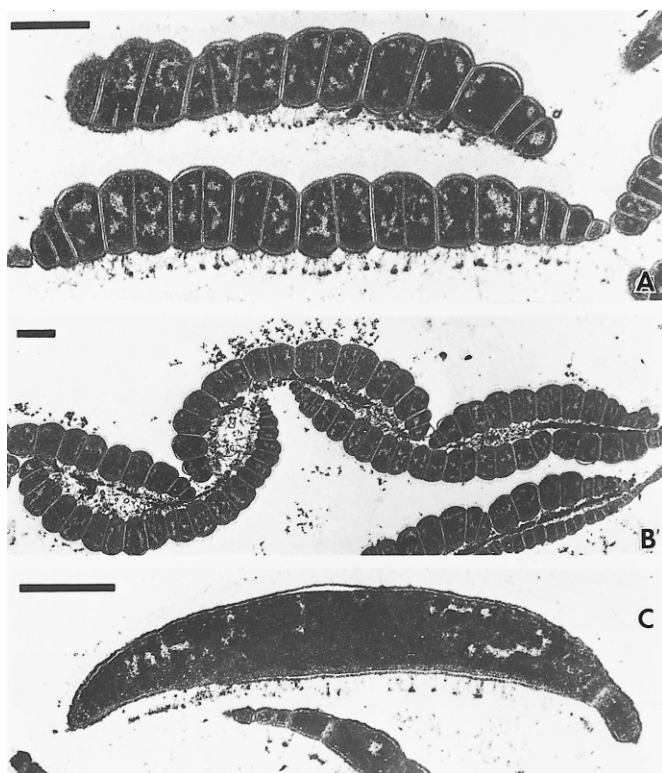

FIGURE BXII.β.77. Scanning electron micrograph of a culture of *S. steedae* ATCC 27411 growing on BSTSY agar (see Enrichment and Isolation Procedures). The depressed tracks where the organism has glided over the surface of the agar are clearly shown. The dorsal surface of the filaments are covered with a capsular material that obscures the individual cells. (Micrograph taken by J. Pangborn.) (Reproduced with permission from J. Pangborn et al., Archives of Microbiology *113:* 197–204, 1977, ©Springer-Verlag.)

FIGURE BXII.β.78. Transmission electron micrograph of thin sections of *S. steedae*. The multicellular nature of the filaments and the dorsal-ventral differentiation are shown. (*A*) Strain ATCC 27411 from an agar surface. (*B*) Strain ATCC 27396 showing a curvature of the filaments that often causes them to stand on their edge in a colony. (*C*) Section of strain ATCC 27411 cut perpendicular to the long axis of the filament. The dorsal-ventral, convex–concave curvature that results in a crescent-shaped transverse section is shown. Notice the fine fibrillar structures on the ventral surface of all of the filaments. Bars = 1 μm. (Micrograph taken by J. Pangborn.) (Reproduced with permission from J. Pangborn et al., Archives of Microbiology *113:* 197–204, 1977, ©Springer-Verlag.)

TABLE BXII.β.96. Characteristics differentiating the species of the genus *Simonsiella*[a]

Characteristic	S. muelleri	S. crassa	S. steedae
Acid from:			
Glucose	+	+	−
Maltose	+	+	−
Trehalose	−	+	−
Ribose	−	+	−
Fructose	−	+	−
Sucrose	−	+	−
Mannitol	−	+	−
Growth at:			
27°C	+	+	−
43°C	−	+	−
pH 6.0	+	+	−
pH 8.0	−	+	−
Source	Humans	Sheep	Dogs

[a]Symbols: +, 90% or more of strains are positive; −, 90% or more of strains are negative

1974). Children possibly have a higher incidence than adults (Fellinger, 1924; Richardson et al., 1966). In dogs and cats *Simonsiella* are common and abundant, the incidence approaching 100% in specimens from the palate or from the buccal cavity (Nyby et al., 1977). In specimens from oral cavities and gingival margins, simonsiellas are less likely to be found (~20% incidence; Saphir and Carter, 1976; Bailie et al., 1978). The incidence varies not only with the site in the mouth from which the specimens are obtained, but also with the method of observation. Direct microscopy yields a higher incidence than cultivation, and microscopic observation of oral specimens growing on plates gives a higher yield than the detection of macroscopic colonies after prolonged incubation (Richardson et al., 1966; Saphir and Carter, 1976; Bailie et al., 1978). Even though these bacteria have been considered as members of the normal flora (Kuhn et al., 1974), they are often underrecognized or not even mentioned as part of the oral microflora. Pathological changes of disease have not been associated with the presence of *Simonsiella* in the mouth. In fact, the incidence of *Simonsiella* in human patients with obvious oral pathology has been considerably lower than in groups of healthy people (Simons, 1922; Bruckner and Fahey, 1969). Despite their low pathogenicity, *Simonsiella* spp. have been isolated from erosive lesions of the human oral cavity (Carandina et al., 1984) and from the gastric aspirate of a neonate (Whitehouse et al., 1987).

Phylogeny and classification Steed designated the family *Simonsiellaceae* to include *Simonsiella* and the morphologically similar genus *Alysiella* (Steed, 1962). Rossau and co-workers demonstrated close relationships between *Simonsiella* strains and strains of *Neisseria, Kingella, Eikenella,* and *Alysiella* by DNA–rRNA hybridization and suggested that *Simonsiella* should be included in the emended family *Neisseriaceae* (Rossau et al., 1989). Dewhirst et al. (1989) found that the type strain of *S. muelleri* by 16S rRNA sequence analysis clustered within the *Neisseriaceae* in the *Betaproteobacteria*. Appropriately, these authors also supported the emended family *Neisseriaceae* to include genus *Simonsiella*.

Kuhn et al. (1977, 1978) have isolated nearly 50 strains of *Simonsiella* from dogs, cats, sheep, and humans. Based on morphology, physiology, and the mol% G + C of the DNA, the strains can be separated into distinct groups that correlated with their source of origin. Three of these groups (dogs, sheep, and humans) have been designated as separate species, while the isolates from cats and other vertebrates need additional characterization

in 2% NaCl. No strains hydrolyze starch or agar or produce urease or indole. No strains produce acid from cellobiose, dulcitol, erythritol, galactose, glycerol, inositol, lactose, mannose, melibiose, melizitose, raffinose, rhamnose, salicin, sorbitol, sorbose, or xylose. Some species may produce acid from other carbohydrates. A rich medium is best for growth, and the addition of serum to the medium has been found to be necessary for some strains and to be advantageous for all other strains (Kuhn et al., 1978).

The cell-bound fatty acid profiles of 48 strains of *Simonsiella* matched the general pattern of Gram-negative bacteria, in which high percentages of even-numbered saturated and monounsaturated fatty acids occur (Jenkins et al., 1977). Tetradecanoic acid was the predominant saturated fatty acid followed by hexadecanoic acid. 9-Hexadecanoic and 9-octadecanoic acids were the predominant monounsaturated fatty acids. Results of stepwise discriminant analysis of the mean relative percentages of tetradecanoic, hexadecanoic, and 9-octadecanoic acids demonstrated that 85% of the isolates (with two cat strains and one dog strain being the exceptions) were correctly identified in their source-of-origin groups.

Habitat In healthy human populations, the incidence of *Simonsiella* is in the range of 30–40% (Simons, 1922; Kuhn et al.,

TABLE BXII.β.97. Selected phenotypic features of *Simonsiella* and *Alysiella* species[a,b]

Characteristic	*A. filiformis* ATCC 15532[T]	*S. crassa* ATCC 15533[T]	*S. muelleri* ATCC 29453[T]	*S. steedae* ATCC 27409[T]
Number of strains investigated	1	2	2	3
Corroding colonies	−	−	−	−
β-Hemolysis	−	+	W	+
Fructose	−	+	+	W
Galactose	−	−	−	−
Glucose	+	+	+	−
Maltose	−	−	D	−
Malonate	+	+	+	−
Mannose	−	−	−	−
Sucrose	+	+	−	−
Trehalose	−	+	−	−
OF test (glucose)	O	O	O	O,W
H$_2$S production	−	−	−	−
Catalase	+	+	+	+
Nitrate reduction	D	+	+	+
Nitrite reduction	−	+	+	−
Phosphatase (alkaline)	−	−	−	−
Gelatine liquefaction	+	+	−	−
Indole production	−	−	−	−
Arginine dihydrolase	−	−	−	−
Lysine decarboxylase	−	−	−	−
Ornithine decarboxylase	−	−	−	−
Glutamyltransferase	−	−	−	−

[a]Symbols: +, positive result; −, negative result; W, weak result; D, different results with different strains; O, oxidative reaction; F, fermentative reaction.

[b]All strains were positive for TMPD-oxidase. All strains were negative for arabinose, dextrose, esculin, lactose, raffinose, rhamnose, salicin, sorbose, starch hydrolysis, xylose, citrate, urease, ONPG test, adonitol, dulcitrol, inositol, and mannitol.

TABLE BXII.β.98. Semiquantitative determination of enzymatic activities in *Simonsiella* and *Alysiella* species using the API-ZYM system (selected from Heiske and Mutters, 1994)[a,b]

Characteristic	*A. filiformis* ATCC 15532[T]	*S. crassa* ATCC 15533[T]	*S. muelleri* ATCC 29453[T]	*S. steedae* ATCC 27409[T]	*Simonsiella* sp. 1 (ATCC 29466)	*Simonsiella* sp. 2 ("*S. kuhniae*" ATCC 27381)	*Simonsiella* sp. 3 (HIM 942-7)
Number of strains investigated	1	2	2	3	1	1	1
Phosphatase, alkaline	1	1	1	1	1	1	1
Esterase (C$_4$)	3	3	3	3	2	3	3
Esterase lipase (C$_8$)	2	2	2	2	2	2	2
Leucine arylamidase	2	2	2	3	2	5	2
Valine arylamidase	1	1	1	1	1	2	1
Cysteine arylamidase	1	1	1	1	1	1	1
Phosphatase (acidic)	1	1	1	1	1	1	1

[a]All strains were positive: naphthol-AS-BI-phosphohydrolase. Tests negative for all strains: lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase.

[b]0, negative reaction; 1, very weak reaction; 2, weak reaction; 3, strong reaction; 5, very strong reaction.

before any decisions about species designations can be made (Kuhn et al., 1978; Kuhn and Gregory, 1978). Numerical taxonomy studies showed that most of the *Simonsiella* strains grouped according to the mammalian host from which they were isolated (Kuhn et al., 1978; Heiske and Mutters, 1994) (Tables BXII.β.96, BXII.β.97, BXII.β.98, BXII.β.99, and BXII.β.100). *Simonsiella* isolates from humans and sheep were each monophyletic in a dendrogram derived from the numerical taxonomy data. Isolates from cats and dogs tended to cluster together with strains from the same host; however, neither group was strictly monophyletic based on the phenotypic data. These observations led to the proposal that each of these mammals hosts a unique type of *Simonsiella* (Kuhn et al., 1978). Kuhn suggested that these host groups represented ecospecies: species of bacteria that each occupied a niche in a unique ecosystem, in this case the mouths of different animals. Accordingly, three of the *Simonsiella* groups were assigned to separate species. *S. muelleri*, *S. crassa*, and *S. steedae* were proposed for *Simonsiella* strains native to humans,

sheep, and dogs, respectively. Hedlund and Staley assessed the relationships between *Simonsiella* strains and other members of the *Neisseriaceae* further by 16S rDNA sequence analysis of 16 *Simonsiella* strains (Hedlund and Staley, 2002) (Fig. BXII.β.79). Most *Simonsiella* strains grouped according to established *Simonsiella* species designations and the mammalian hosts from which they were isolated. The host groups corresponded to the three existing *Simonsiella* species— *S. muelleri*, *S. steedae*, and *S. crassa* —which are commensals of humans, dogs, and sheep, respectively. The fourth group consisted of *Simonsiella* isolates from domestic cats, and the phenotypic results previously obtained by Kuhn were common to the *Simonsiella* cat isolates (Kuhn et al., 1978). Although this entity was phylogenetically more diverse than the other groups and phenotypically similar to *S. steedae*, the distinct phylogeny and ecological habitat of this group supported the establishment of a new species. Thus, the phylogeny together with phenotypic data supported the suggestion of "*S. kuhniae*" to encompass *Simonsiella* strains isolated from domestic

TABLE BXII.β.99. Results of the API 20NE test and selected reaction of the API rapid ID 32A test activities in *Simonsiella* and *Alysiella* species (from Heiske and Mutters, 1994)[a,b]

Characteristic	*Alysiella filiformis* ATCC 15532[T]	*S. crassa* ATCC 15533[T]	*S. muelleri* ATCC 29453[T]	*S. steedae* ATCC 27409[T]
Number of strains	1	2	2	3
Nitrate reduction	W	+	+	+
Indole production	−	−	−	−
Glucose acidification	+	+	+	+
Arginine dihydrolase	−	−	−	−
Gelatin hydrolysis	−	+	−	−
Assimilation of:				
Glucose	+	+	+	−
Mannose	−	−	−	−
N-Acetylglucosamine	−	−	−	−
Maltose	−	−	−	−
Gluconate	−	−	−	−
Malate	−	−	+	−
Arginine arylamidase	+	+	+	+
Proline arylamidase	−	−	−	−
Leucylgycine arylamidase	+	+	+	+
Phenylglycine arylamidase	−	+	−	−
Leucine arylamidase	+	+	+	+
Tyrosine arylamidase	+	+	W	W
Alanine arylamidase	+	+	+	+
Glycine arylamidase	+	+	+	+
Glutamic acid decarboxylase	+	−	+	−
Glutamylglutamate arylamidase	+	−	+	−
Serine arylamidase	+	+	+	+

[a]Symbols: +, positive result; −, negative result; W, weak result.

[b]Test negative with all strains: assimilation of adipate, arabinose, caprate, citrate, mannitol, phenyl acetate; hydrolysis of esculin; indole production; urease.

cats, as proposed by Hedlund and Staley (2002). Hedlund and Staley (2002) have provided evidence that the different *Simonsiella* strains may have co-evolved with their respective hosts. The bacteria seem to be evolving more rapidly than their hosts, most likely because they are growing more quickly (Interview with J.T. Staley in Stencel, C. Microbial diversity: Eyeing the big picture. ASM News 66:146, 2000). These considerations indicate that one needs to look beyond 16S rDNA for phylogenetic analysis of these species.

Members of the genus *Simonsiella* possess a morphology that is striking and unique among bacteria. For this reason it could be expected that they would make up a coherent phylogenetic group that excluded other bacteria. However, the *Simonsiella* are polyphyletic in 16S rDNA based phylogenetic analysis (Fig. BXII.β.61 in Chapter *Neisseriales*; Hedlund and Staley, 2002). Such instabilities may be attributable to horizontal gene transfer between members of the *Neisseriaceae*, including *Simonsiella*. Most members of the family *Neisseriaceae* are naturally competent for transformation (Bøvre, 1980). The discrepancies between 16S rDNA phylogenies of the genus *Neisseria* and those derived from analyses of other loci (Smith et al., 1999b) or from chemotaxonomic data (Barrett and Sneath, 1994) have suggested that the irregularities in the phylogenetic trees were due to interspecies gene exchange and that certain *Neisseria* 16S rDNA sequences are hybrids (Zhou et al., 1997; also see chapter on Genus *Neisseria*). Thus, members of the genus *Neisseria* could have acquired and recombined with *Simonsiella* 16S rDNA sequences. It is not known whether *Simonsiella* strains themselves are competent; if they are, the horizontal gene transfer from *Neisseria* or other oral flora to *Simonsiella* could have added to the phylogenetic complexity.

ENRICHMENT AND ISOLATION PROCEDURES

At the present time, there are no enrichment procedures for *Simonsiella*. Isolation directly from the oral cavity has been achieved by several people, however. The easiest method for isolation of *Simonsiella* was described by Kuhn et al. (1978). A sterile cotton swab is rubbed over the palate, tongue, or inner surface of the cheeks of the animal and immediately rolled over the surface of a thin layer of BSTSY agar (tryptic soy broth without dextrose (Difco), 27.5 g; yeast extract (Difco), 4 g; agar, 15.0 g; water, 900 ml; and sterile bovine serum, 100 ml, which is added after autoclaving and cooling the other ingredients to 45°C) in a plastic Petri dish. Without delay, the dish is then placed into a 37°C incubator for about 6–10 h. During this short incubation period the filaments glide away from the oral epithelial cells and multiply. The Petri dish is then scanned via a microscope with a magnification of up to ×125 or a dissecting microscope. Isolated filaments or microcolonies are picked from the agar surface and transferred to fresh plates of BSTSY agar. Suitable instruments for transfer could include a dissecting needle, inoculating needle, toothpick, dental probe, light bulb filament, or other fine-tipped instrument. Macroscopically visible colonies generally appear within 16–24 h after transfer. Although this method was used to isolate nearly 50 strains of *Simonsiella*, some strains did not grow on this medium and could not be isolated.

Steed (1962) isolated *Simonsiella* from sheep with a medium consisting of Oxoid nutrient agar plus 10% horse or ox serum. This medium was not suitable for the isolation of strains from humans (Kuhn et al.,1978). Berger (1963) used blood agar to detect and isolate *Simonsiella*, but this medium suffers from the opaqueness of the blood, which prevents easy observation of the colonies with a microscope.

TABLE BXII.β.100. Cellular carbohydrate patterns in *Simonsiella* and *Alysiella* species (selected from Heiske and Mutters, 1994)

	Characteristic	*A. filiformis* ATCC 15532[T]	*S. crassa* ATCC 15533[T]	*S. muelleri* ATCC 29453[T]	*S. steedae* ATCC 27409[T]	*Simonsiella* sp. 1 (ATCC 29466)	*Simonsiella* sp. 2 ("*S. kuhniae*") (ATCC 27381)	*Simonsiella* sp. 3 (HIM 942-7)
RT (min)	Carbohydrate	1[a]	2	2	3	1	1	1
11.69	*Meso*-crythrol	0[b]	3	0	0	0	3	0
12.02	*Meso*-crythrol	4	3	0	0	0	0	0
12.1	Ribose/lyxose (A)[c]	5	5	5	4	5	5	5
12.14	Rhamnose (A)	0	0	0	0	0	0	0
12.5	Fucose (A)	0	0	0	0	0	0	0
13.08	Fucose (O)	0	0	0	0	0	0	0
13.31	Ribose/lyxose (O)	5	5	5	5	4	5	5
13.36	Rhamnose (O)	0	0	0	0	0	0	0
13.64	Ribose/lyxose (O)	7	8	8	8	7	7	7
13.68	Rhamnose (O)	0	0	4	2	2	2	3
13.72	Fucose (O)	0	0	0	0	0	0	0
13.84	Threose	0	0	0	0	0	0	0
13.92	Arabinose (O)	2	1	0	1	2	0	2
15.39	C-6	0	1	2	3	2	3	2
15.46	C-6	2	0	0	3	3	3	2
15.5	C-6	0	2	3	3	2	0	0
15.54	Glucose (A)	3	3	3	4	3	3	3
15.82	Mannose	0	2	0	0	0	0	0
15.86	Galactose (A)	3	3	3	3	2	2	3
14.34	Galactose (O)	3	3	4	3	3	3	3
16.62	Glucose (O)	3	3	3	3	3	2	3
16.75	Mannose (O)	0	2	0	1	1	0	2
16.8	Glucose (O)	5	5	4	5	6	4	5
16.9	Galactose (O)	5	4	6	5	4	4	6
17.04	Sorbose/tagatose	2	0	0	1	2	1	0
17.19	Glucosamine (A)	4	5	5	5	4	4	4
17.3	Inositol	1	0	0	0	1	3	2
17.75	Lyxose (phenyl) (A)	2	2	2	1	0	2	3
18.08	Galactosamine (A)	1	2	3	3	2	0	0
18.18	Heptose (A)	0	1	0	2	0	0	0
18.24	Muramic acid	2	3	3	3	4	4	3
18.4	Heptulose (A)	0	0	0	0	0	0	0
18.42	Glucosamine (O)	3	3	3	3	3	2	2
18.58	Glucosamine (O)	3	5	4	4	5	4	4
18.77	Galactosamine (O)	3	3	4	4	3	2	3
18.93	Heptose (O)	0	1	0	2	0	0	0
19.18	Glucoheprose (O)	1	3	2	3	1	0	0
19.48	Glucoheprose (O)	3	2	2	3	0	2	1
19.57	Muarmic acid	1	2	2	2	2	2	0
20.14	Talose (phenyl) (A)	0	0	1	0	0	0	0

[a]Number of strains tested.

[b]% of total carbohydrate amounts: 0, absent; 1, 0.1–0.5%; 2, 0.51–1.0%; 3, 1.1–3.0%; 4, 3.1–6.0%; 5, 6.1–10.0%; 6, 10.1–20.0%; 7, 20.1–30.0%; 8, 30.1–50.0% (RT value of *n*-ocradecane, 14.11).

[c](A), peracetylated aldononitrile; (O), peracetylated O-methyloxime; C-6, unknown carbohydrate with six C atoms; phenyl, prenylated carbohydrate; no specification of reaction type, only acetylation could be observed (in case of sugar alcohols, amino sugars, and similar).

MAINTENANCE PROCEDURES

Freshly isolated cultures should be grown at 37°C and transferred about every 2–3 d to the same medium on which they were isolated. Older cultures must be transferred at intervals of about 1 week.

Refrigeration of cultures is not recommended for preservation, but they can be preserved by freezing in liquid nitrogen using glycerol as a cryoprotectant or by lyophilization.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

The procedures for characterizing *Simonsiella* were first described by Steed (1962) and later modified by Kuhn et al. (1978). The procedures generally parallel those that are commonly used for other taxa, except that for *Simonsiella* most media contain 10% serum. All incubations are carried out at 37°C. The procedures below are from Kuhn et al. (1978).

Casein hydrolysis BSTSY agar containing 2% skim milk is inoculated, incubated for 3 d, and examined for evidence of hydrolysis.

Starch hydrolysis TSY agar (BSTSY agar without serum) containing 5% soluble starch is inoculated, incubated for 4 d, and then flooded with Lugol's iodine.

Gelatin hydrolysis TSY broth containing 10% serum and 12% gelatin is inoculated and tested for liquefaction after 5 d of incubation by chilling the tube to 4°C.

Peptonization of litmus milk Litmus milk (Difco) containing 10% serum is inoculated and examined for peptonization over a 14-d incubation period.

Action on carbohydrates A medium is poured into Petri dishes, inoculated, and examined after 48 h for the production of acid (yellow color). It consists of the following ingredients:
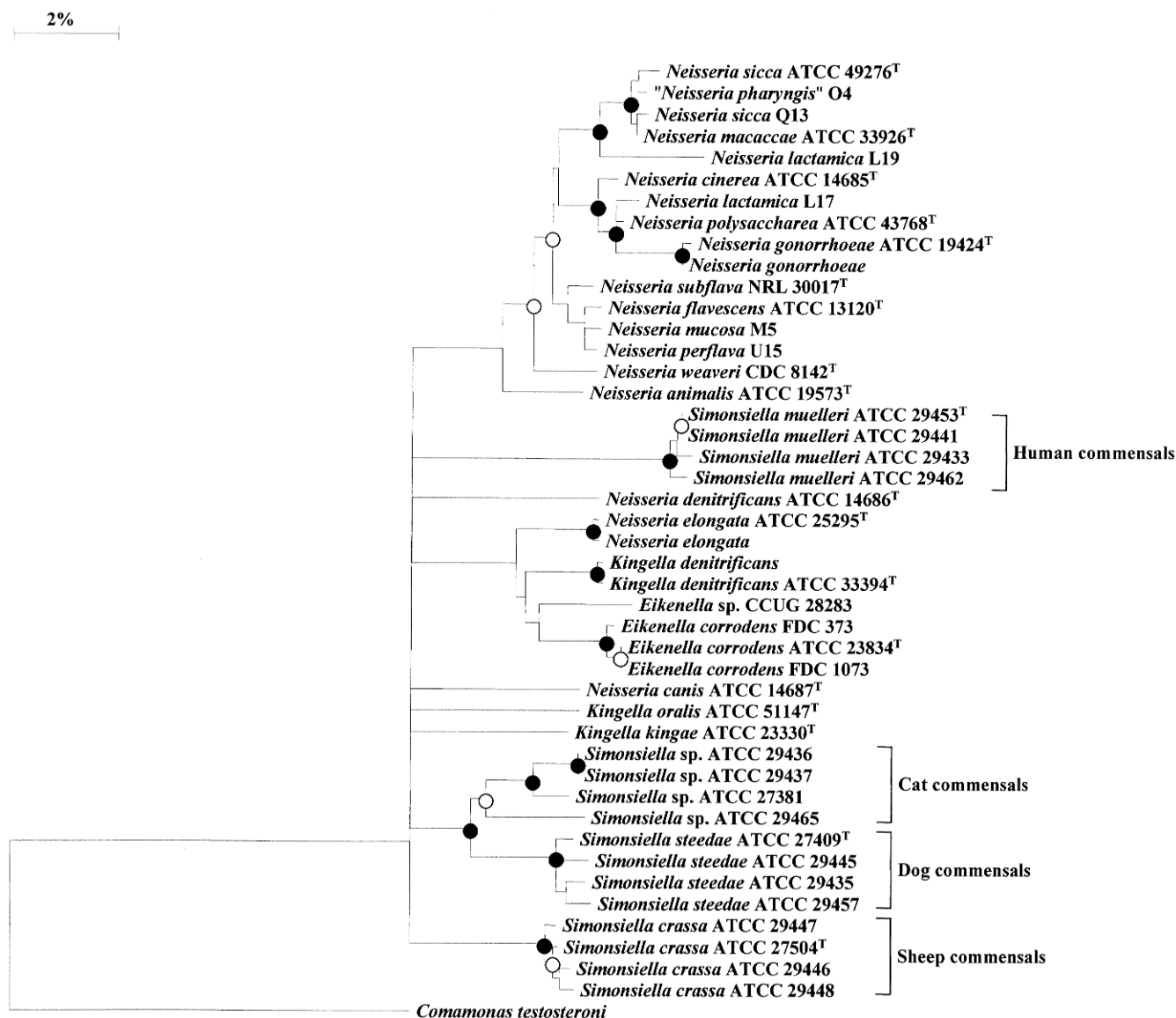
FIGURE BXII.β.79. Phylogenetic neighbor-joining tree of the genus *Simonsiella* of the family *Neisseriaceae* based on nucleotide sequence of the 16S rRNA genes. *Comamonas testosteroni* was used as an outgroup. ●, Nodes with >90% bootstrap support for all analyses; ○, with >75% bootstrap support. Values below 50% are not shown. Bar = 2% nucleotide divergence. (Reproduced with permission from B.P. Hedlund and J.T. Staley, Journal of Systematic and Evolutionary Microbiology 52: 1377–1382, 2002, ©International Union of Microbiological Societies.)

phenol red broth (Difco), 1.6%; yeast extract (Difco), 0.2%; agar (Difco), 1.5%; phenol red (Hartman Leddon Co., Philadelphia, Pennsylvania), 0.007%; serum, 10%; and carbohydrate, 1%.

Indole production BSTSY broth containing 1% tryptone is inoculated, incubated for 3 d, and examined for evidence of indole production with Kovac's reagent.

Nitrate reduction TSY broth containing 0.2% KNO$_3$ in a Durham tube assembly is inoculated and examined for 5 d by the dimethyl-α-naphtholamine/sulfanilic acid method (Miller and Neville, 1976).

DIFFERENTIATION OF THE GENUS *SIMONSIELLA* FROM OTHER GENERA

The unusual morphology of the *Simonsiella* filament serves to differentiate the genus from all other procaryotic organisms. *Aly-siella* resembles *Simonsiella*, but filaments of *Alysiella* do not show the unusual dorsal-ventral differentiation and do not have rounded ends, and the individual cells are paired within the filament.

Caryophanon filaments may be mistaken for *Simonsiella* in stained preparations. However, in live preparations the cylindrical form of *Caryophanon* and the flattened shape of *Simonsiella* are easily distinguished. Also, *Caryophanon* are motile by flagella; they do not glide.

TAXONOMIC COMMENTS

The citation "Schmid *in* Simons" following the generic name in the title is the result of Simons (1922) crediting the name *Simonsiella* to G. Schmid. However, no record can be found that Schmid published the name *Simonsiella*.

When Simons (1922) first described the genus *Simonsiella*

from the human oral cavity, he named two species *S. muelleri* and *S. crassa*, which he differentiated based on the width of the filaments. Later, Steed (1962), examining strains from sheep, and Berger (1963), examining strains from guinea pigs, named their isolates *S. crassa* and *S. muelleri*, respectively, because of the similarity in filament width to those known species. When Kuhn et al. (1978) carried out an extensive investigation of 49 isolates from humans, dogs, cats, and sheep, they determined that each animal had its own species and that filament width was not a suitable indicator for the differentiation of species.

Simonsiella has been reported from the oral cavity of many warm-blooded vertebrates including cats, chickens, cows, dogs, goats, guinea pigs, humans, horses, pigs, rabbits, and sheep. Only those from cats, dogs, humans, and sheep have been described in detail (Steed, 1962; Kuhn et al., 1978). When those from other animals are sufficiently characterized, it may be necessary to revise the number of species within this genus and the means of differentiation among the species. Moreover, some *Simonsiella* have been refractory to isolation, possibly indicating that they were a different strain or species (Kuhn et al., 1978) from those that grew and were isolated on BSTSY agar.

Kuhn et al. (1978) characterized several *Simonsiella* strains from cats. 16S rDNA sequence analysis of *Simonsiella* strains indicated that they are members of the family *Neisseriaceae* (Hedlund and Staley, 2002).

FURTHER READING

Heiske, A. and R. Mutters. 1994. Differentiation of selected members of the family *Neisseriaceae* (*Alysiella*, *Eikenella*, *Kingella*, *Simonsiella* and CDC groups EF-4 and M-5) by carbohydrate fingerprints and selected phenotypic features. Zentbl. Bakteriol. *281*: 67–79.

Kuhn, D.A. 1981. The genera *Simonsiella* and *Alysiella*. *In* Starr, Stolp, Trüper, Balows and Schlegel (Editors), The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria, Springer-Verlag, New York. pp. 390–399.

McCowen, R.P., K.J. Cheng and J.W. Costerton. 1979. Colonization of a portion of the bovine tongue by unusual filamentous bacteria. Appl. Environ. Microbiol. *37*: 1224–1229.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *SIMONSIELLA*

Some differential features of the four recognized species of *Simonsiella* are shown in Tables BXII.β.96, BXII.β.97, BXII.β.98, BXII.β.99, and BXII.β.100.

List of species of the genus Simonsiella

1. **Simonsiella muelleri** Schmid *in* Simons 1922, 504[AL]

   muel′ le.ri. M.L. gen. n. *muelleri* Müller, named for R. Müller, who first described these organisms.

   See Tables BXII.β.96, BXII.β.97, BXII.β.98, BXII.β.99, and BXII.β.100 and the generic description for many features. The long axis of the cells (width of filament) varies from 2.1 to 3.5 μm, with an average of about 2.5–3.2 μm; the short axis (length of individual cell) varies from about 0.5 to 0.9 μm, with an average of about 0.8 μm. Casein, gelatin, esculin, and hippurate are not hydrolyzed. No change occurs in litmus milk. The ability to reduce nitrates varies among strains, with about half of them being positive; when reduction occurs, nitrite is produced, and a few strains produce N_2 gas. H_2S production is variable and inconsistent. Found in the oral cavity of humans.

   The mol% G + C of the DNA is: 40–42 (Bd) for 16 of 18 strains; two other strains had a G + C mol% 44 and 50 (Bd).

   Type strain: ATCC 29453, CCUG 30554, CIP 103436, DSM 2579, ICPB 3636, LMG 7828.

   GenBank accession number (16S rRNA): M59071, AF328147.

2. **Simonsiella crassa** Schmid *in* Simons 1922, 504[AL]

   cras′ sa. L. fem. adj. *crassa* thick.

   See Tables BXII.β.96, BXII.β.97, BXII.β.98, BXII.β.99, and BXII.β.100 and the generic description for many features. The long axis of the cells (width of filament) varies from 1.9 to 3.6 μm, with an average of about 2.9–3.5 μm; the short axis (length of individual cell) varies from 0.7 to 0.9 μm, with an average of about 0.8 μm. Steed (1962) reported that her isolates produced acid from inulin and

that four of six isolates produced acid from arabinose. Kuhn et al. (1978) reported negative results with both carbohydrates for her isolates, as well as for one of Steed's strains. The discrepancy may be due to differences in the method of testing. Gelatin and casein are hydrolyzed. Litmus milk is peptonized. Nitrate is reduced to nitrogen gas. MR-negative. VP-negative. Steed reported that all of her isolates produced H_2S, but Kuhn et al. (1978) stated that H_2S production is variable and inconsistent. Found in the oral cavity of sheep.

   The mol% G + C of the DNA is: 44–45 (Bd).

   Type strain: ATCC 27504, CCUG 25927, CIP 103341, DSM 2578, ICPB 3651, LMG 782, NCTC 10283.

   GenBank accession number (16S rRNA): AF328141.

3. **Simonsiella steedae** Kuhn and Gregory 1978, 13[AL]

   stee′ dae. M.L. gen. n. *steedae* of Steed; named for P. Steed (Glaister) who first isolated axenic cultures of *Simonsiella* and erected the family *Simonsiellaceae*.

   See Tables BXII.β.96, BXII.β.97, BXII.β.98, BXII.β.99, and BXII.β.100 and the generic description for many features. The long axis of the cells (width of filament) varies from 2.5 to 7.1 μm, with an average of about 3.1–3.8 μm; the short axis (length of individual cell) varies from 0.7 to 1.3 μm, with an average of about 1.1 μm. Casein, gelatin, esculin, and hippurate are not hydrolyzed. No change in litmus milk. Most strains reduce nitrate to nitrite without gas production. H_2S production is variable and inconsistent. Found in the oral cavity of dogs.

   The mol% G + C of the DNA is: 48–52 (Bd).

   Type strain: ATCC 27409, CCUG 30555, CIP 103435, DSM 2580, ICPB 3604, LMG 7830.

   GenBank accession number (16S rRNA): AF328153.

4. "Simonsiella kuhniae"

kuhn.i.ae. F.L. gen. n. *kuhniae* of Kuhn, named after Daisy A. Kuhn, in honor of her work on the isolation, characterization, and autecology of *Simonsiella* in domestic animals and humans.

Multicellular filaments, segmented into groups of 8–12 cells; cells possess dorsal-ventral asymmetry, gliding on ventral surface. Cell width 2.7–4.4 μm; cell length 0.5–1.1 μm; cell thickness 0.5–1.1 μm. Terminal segments often decreased in size. Colonies are entire, low convex <1–2 mm diameter, smooth and butyrous, opaque with pale, nondiffusible yellow pigmentation. Strict aerobe. Catalase and oxidase positive. Chemoorganotrophic. Acid not produced from D-glucose, maltose, D-ribose, D-fructose, sucrose, mannitol, salicin. Weakly proteolytic on casein. No activity on gelatin, inspissated serum, or litmus milk. Growth at 37°C, pH 7.2, and pH 8.0; no growth at 27°C, 43°C, or pH 6.0. Nitrate may or may not be reduced. Found in the oral cavity of domestic cats, *Felis domesticus.*

Deposited strain: ATCC 29436, ICPB 3618.

GenBank accession number (16S rRNA): AF328149.

Additional Remarks: Strains ATCC 29437 (ICPB 3619), ATCC 27381 (ICPB 3601), and ATCC 29465 (ICPB 3648) also belong to this species.

Genus XII. **Vitreoscilla** *Pringsheim 1949c, 70*[AL]

William R. Strohl

Vit.re.os.cil' la. L. adj. *vitreus* glassy, clear; L. n. *oscillum* a swing; M.L. fem. n. *Vitreoscilla* transparent oscillator.

Cylindrical cells (two species) or sausage-shaped cells (one species) ranging from 1.0 to 3.0 μm in diameter. The organisms occur as **colorless filaments** with lengths up to several hundred μm. Two species have cells that are not normally visible within filaments; one species has cells that are clearly visible within the filaments. **Gram negative. Gliding motility.** No locomotor organelles known. **Resting stages are not known. Sheaths and holdfasts are not produced. Nonpigmented. Aerobic to microaerophilic**, having a respiratory type of metabolism with oxygen as the terminal electron acceptor. No growth anaerobically. **Sulfur inclusions are not formed from hydrogen sulfide or thiosulfate. Chemoorganotrophic.** Two species are oligotrophic and grow best at low nutrient concentrations; one species can grow luxuriantly on rich media, e.g., 0.5% peptone broth. Various organic acids and amino acids are used as carbon and energy sources. Found in dung, soil, water with decaying plant material, sediments, and in association with oscillatorian mats.

The mol% G + C of the DNA is: 42–63.

Type species: **Vitreoscilla beggiatoides** Pringsheim 1949c, 70, emend. Strohl, Schmidt, Lawry, Mezzino and Larkin 1986a, 311.

Further descriptive information

Morphological characteristics The genus *Vitreoscilla* consists of two morphological types. One type is characterized by *V. beggiatoides* (the type species) and *V. filiformis.* The cells are ultrastructurally similar to those of *Beggiatoa* in having (i) continuous outer layers of the filaments (Figs. BXII.β.80 and BXII.β.81), (ii) extra cell wall layers outside of the "lipopolysaccharide-like" layer (Strohl et al., 1986a), (iii) large accumulations of poly-β-hydroxybutyrate (PHB; sometimes >50% of dry cell mass), and (iv) membrane invaginations in the cytoplasm. If these filaments are mechanically broken up into individual cells, the cells die—an indication that these are truly multicellular bacteria like the beggiatoas. The cells within a filament of these species divide via septation, with only the cytoplasmic membrane and peptidoglycan layers invaginating, like the closure of an iris diaphragm, as in *Beggiatoa* (Strohl and Larkin, 1978a). In thin sections observed by transmission electron microscopy, cells of *V. beggiatoides* and *V. filiformis* appear similar to cells of *Beggiatoa* (Strohl et al., 1986a). Furthermore, the sacrificial-cell-death life cycle described for beggiatoas (Strohl and Larkin, 1978a) appears to apply to *V. beggiatoides* as well, and may also occur with *V. filiformis* strains.

The second morphological type of *Vitreoscilla* is exemplified by *V. stercoraria.* Cells of this species have discontinuous outer layers (Figs. BXII.β.82 and BXII.β.83), and the filaments appear similar to chains of bacilli. The cells are held together by a ruthenium-red-staining material, but they can be mechanically broken apart without accompanying massive cell death. Multicellular filaments are formed seemingly only because the cells do not completely detach after division. Reports have indicated that certain growth conditions promote growth of *V. stercoraria* as single

FIGURE BXII.β.80. Thin-section electron micrograph of *V. beggiatoides* strain B23SS showing the continuous cell wall and extra cell layers. Also note the large depositions of PHB. Bar = 0.5 μm. (Reproduced with permission from W.R. Strohl et al., International Journal of Systematic Bacteriology *36:* 302–316, 1986, ©International Union of Microbiological Societies.)
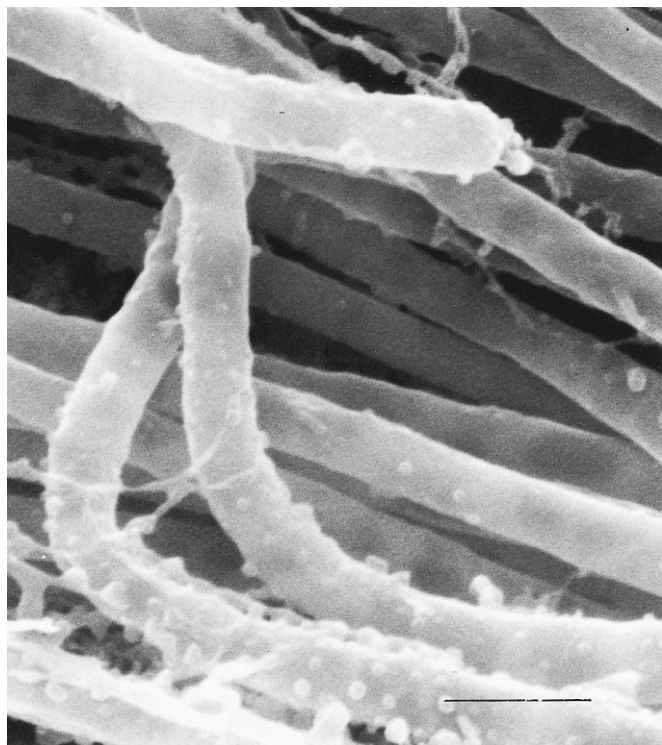
FIGURE BXII.β.81. Scanning electron micrograph of *V. beggiatoides* strain B23SS, with continuous cell envelope and slime-like matrix shown. Bar = 5 μm.
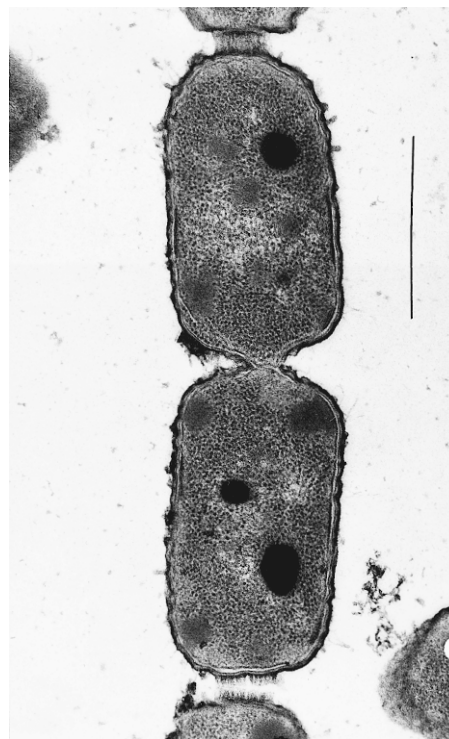

FIGURE BXII.β.82. Thin-section micrograph of ruthenium-red-stained *V. stercoraria* VT-1, with chains of cells held together by connecting material, and the Gram-negative cell envelope shown. Bar = 1 μm.

cells (Brzin, 1966a, b). Ultrastructurally, *V. stercoraria* cells have a typical Gram-negative cell envelope, with the addition of an external, ruthenium-red-staining surface layer (Fig. BXII.β.82; Strohl et al., 1986a). Perhaps the most notable feature of *V. stercoraria* is the presence in the cells of a bacterial form of hemoglobin (Wakabayashi et al., 1986; Khosla and Bailey, 1988b). The *V. stercoraria* hemoglobin gene has been cloned, sequenced, and characterized in detail, and has been used in recombinant form in several attempts to improve oxygen transfer to industrially significant organisms (Khosla and Bailey, 1988a; Brunker et al., 1998).

Cultural conditions For all *Vitreoscilla* species, the growth conditions determine the shape and characteristics of the colonies. On agar media containing low amounts of nutrients, wavy, curly, or spiral colonies are produced, from which trichomes glide radially outward from the central colony area. On solid media containing high nutrient concentrations, the trichomes spread very little and can form drop-like colonies resembling those of most eubacteria.

Nutritional characteristics *Vitreoscilla* species were formerly differentiated based on trichome size alone, although critical nutritional (Strohl et al., 1986a) and phylogenetic (Stahl et al., 1987; Dewhirst et al., 1989) differences among them are now known. *V. beggiatoides* and *V. filiformis* strains grow best on media with very low nutrient concentrations (e.g., 0.05–0.1% peptone, acetate, etc.), but differ in the nutrients utilized. *V. beggiatoides* can grow on a simple defined medium containing acetate, ammonium, and basal salts and is fairly restrictive in the nutrients it utilizes, i.e., certain C_2–C_5 organic acids and amino acids. *V.*

filiformis can grow on the minimal medium described above, but it also can utilize glucose and citrate. Neither species, however, tolerates high concentrations of nutrients or nutritionally rich media well. Similarly, neither species grows to great cell masses, with 50–80 mg/l of dry cell mass being the approximate maximum. These organisms can withstand moderate concentrations of sulfide, even though they do not form sulfur depositions from it. *V. beggiatoides* B23SS and three *V. filiformis* strains can fix molecular nitrogen, as determined by acetylene reduction (Polman and Larkin, 1990). In all four cases, the presence of a fixed ammonium salt source in the medium abolishes the ability of the organisms to reduce acetylene (Polman and Larkin, 1990).

Vitreoscilla stercoraria differs from the other two species in that it grows luxuriantly on rich media (0.5% peptone, w/v). *V. stercoraria* is an obligate amino-acid-utilizing organism with fairly complex nutritional requirements (Mayfield and Kester, 1972, 1975), although it does not utilize glucose. *V. stercoraria* grows very well in nutritionally complex and rich media, and it achieves much higher cell masses during growth than do the other vitreoscillas.

Metabolic characteristics Cultures of *V. beggiatoides* and *V. filiformis* are able to oxidize both the methyl and carboxyl carbons of acetate to CO_2 (Strohl et al., 1986b). The rate of acetate oxidation is very high, as it is with the beggiatoas. These organisms may use the glyoxylate bypass cycle when growing on acetate, but this has been investigated very little. *V. stercoraria* contains all of the enzymes of the tricarboxylic and glyoxylate bypass cycles (W.R. Strohl and G.W. Luli, unpublished data). The activities of the enzymes isocitrate lyase and malate synthase are stimulated significantly by the addition of 1% sodium acetate to the *V. ster-*
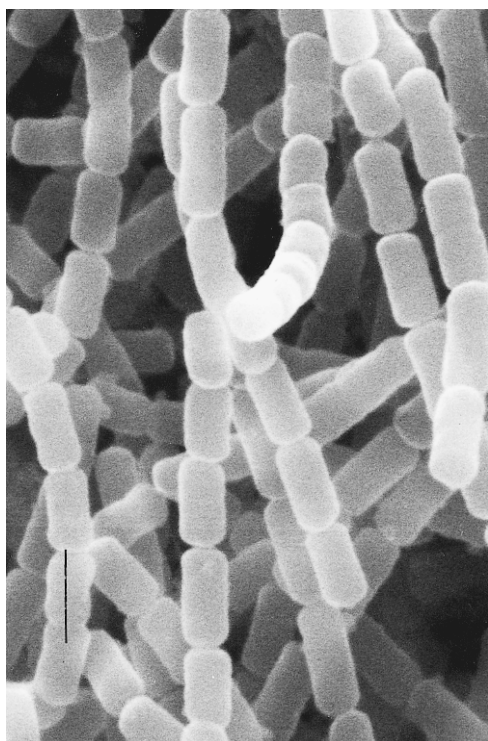
FIGURE BXII.β.83. Scanning electron micrograph of *V. stercoraria* strain VT-1, with the discontinuous cell walls and the connecting material between the cells shown. Bar = 1 μm.

coraria growth medium (W.R. Strohl and G.W. Luli, unpublished data). This is in apparent contradiction to Pringsheim's original statement that acetate does not stimulate growth of *V. stercoraria* (Pringsheim, 1951).

Plasmids *V. beggiatoides* and *V. filiformis* strains are apparently devoid of small plasmids such as the 12.3 and 12.8 × 10⁶ M$_r$ (18.9 and 19.7 kb, respectively) plasmids observed in *Beggiatoa alba* (Minges et al., 1983). Strains of *V. stercoraria* contain small plasmids with an apparent 1.4 × 10⁶ M$_r$ (~2.2 kb; Minges et al., 1983). No functions have yet been ascribed to these plasmids. DNA transformation procedures have been described for *V. stercoraria* (Navani et al., 1996), but not for the trichome-forming vitreoscillas.

Habitat Vitreoscillas of the *V. stercoraria* type are usually found in dung, soil, water with decaying plant material, and in association with oscillatorian mats (Pringsheim, 1949c, 1951). *V. beggiatoides* strain B23SS (group B strains, as described in Strohl and Larkin, 1978b) was isolated from a sandy, lightly sulfide-emanating sediment that also included sulfur-containing beggiatoas.

ENRICHMENT AND ISOLATION PROCEDURES

Enrichment and isolation procedures for *V. beggiatoides, V. filiformis*, and other trichome-forming strains are basically the same as those described in the chapter on the genus *Beggiatoa* (this volume; see also Pringsheim, 1964 and Strohl and Larkin, 1978b). The medium used, however, should reflect the type of *Vitreoscilla* being sought (i.e., lack of sulfide in the media and the type and amount of organic supplements). A medium that is excellent for the isolation and maintenance of *V. beggiatoides* is "MY medium",

described in the article on *Beggiatoa* (this volume), but without the added reduced sulfur source. For *V. filiformis* and similar strains, a modification of MY medium in which glucose or citrate is substituted for acetate might be more appropriate.

For isolation of *V. stercoraria*, small samples of dung can be placed directly onto plates containing a nutritionally rich medium. After several days, trichomes gliding away from the inoculum can be observed with a dissecting microscope. Those filaments can be retrieved with a flame-sterilized 26-gauge needle, as described for *Beggiatoa*. A good medium for this purpose is 0.1-CAYTS medium, which contains 0.1% each of casitone, tryptone, sodium acetate, and yeast extract.

MAINTENANCE PROCEDURES

Vitreoscillas can survive for about three weeks on plates of standard media employed for their growth. In general, the lower the concentration of nutrients in the media, the longer the time of survival. Care should be taken to transfer from the edges of the spreading colonies, so as to transfer the youngest and most active filaments. *V. stercoraria* can be lyophilized. *V. beggiatoides* and *V. filiformis* cannot be lyophilized, but they can be stored at −70°C or −196°C in the presence of 20–30% glycerol.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Because the larger species of *Vitreoscilla* are distinguished from beggiatoas by their inability to deposit sulfur, it is critical that the tests for sulfide oxidation and sulfur deposition be sensitive and reproducible. Investigators frequently have viewed the filaments with a phase microscope and assumed that refractile objects are sulfur. In fact, the strains now included in *V. filiformis* were once considered to be *Beggiatoa* strains because the phase-bright bodies (presumably polyphosphate) in these organisms were interpreted as sulfur (Pringsheim, 1964; Kowallik and Pringsheim, 1966).

One method for determining if inclusions contain sulfur is a chemical analysis of ethanol-extracted (Jørgensen and Fenchel, 1974) or carbon-disulfide-extracted (Nelson and Castenholz, 1981) filaments. A much more sensitive, but more difficult and expensive, method for analyzing sulfur deposition is through the oxidation of ³⁵S-labeled Na₂S at a physiological pH to internal ³⁵S⁰ (Strohl and Schmidt, 1984; Strohl et al., 1986a; Schmidt et al., 1987b). Controls should be included for the adsorption of Na₂³⁵S by autoclaved cells and for Na₂³⁵S assimilation by known sulfide-oxidizing organisms (e.g., *Beggiatoa* species) and non-sulfide-oxidizing organisms (e.g., *V. stercoraria*). Once it is demonstrated that labeled sulfide is assimilated, it should be at least 50–70% extractable by washing with warm ethanol or, alternatively, ~90% extractable by warm ethanol treatment followed by a wash with ethanol/diethyl ether (1:1) (W.R. Strohl, unpublished data).

DIFFERENTIATION OF THE GENUS *VITREOSCILLA* FROM OTHER GENERA

Although the vitreoscillas make up a heterogeneous group, they still can be differentiated from members of other genera (Table BXII.β.101). The primary distinguishing characteristics include their ability to glide but not deposit sulfur, their inability to produce sheaths or holdfasts, and their absence of pigmentation.

TAXONOMIC COMMENTS

Briefly, the three species that currently make up the genus *Vitreoscilla* are not phylogenetically coherent, and it is proposed that

each of the three species within the genus *Vitreoscilla* should be placed into a separate genus. While these organisms share some general features, they are vastly different in morphological, physiological, and phylogenetic characteristics.

Phylogenetic analyses have clarified the taxonomy of these organisms to some degree. Whereas the resemblance of *Vitreoscilla* filaments to those of cyanobacteria caused Pringsheim to label them as colorless forms of cyanobacteria (Pringsheim, 1949c), none of the species is phylogenetically related to any cyanobacterium (Reichenbach et al., 1986; Stahl et al., 1987). Moreover, phylogenetic analysis of the vitreoscillas, beggiatoas, and similar organisms has demonstrated that gliding motility is not a particularly useful taxonomic or phylogenetic marker, as has been pointed out by Stahl et al. (1987). In another example, members of the gliding cytophagas are now grouped together with non-gliding flavobacteria and bacteroides, and, in some instances, former *Cytophaga* species have been reclassified as *Flavobacterium* species (Nakagawa and Yamasato, 1993).

Sequence analysis of 5S rRNA has placed *V. beggiatoides* strain B23SS (the neotype strain) in the γ3-subgroup (Woese, 1987) of the *Gammaproteobacteria* (Stahl et al., 1987) (Fig. BXII.β.84A). The authors state that *V. beggiatoides* is "specifically, but distantly, related to *B. alba*" strain B18LD (type strain), and point out that the evolutionary distance between the two organisms is greater than that separating *E. coli* and *Proteus vulgaris* (Stahl et al., 1987; see also Fig. BXII.β.84A).

Similar 5S rRNA analysis indicates that *V. stercoraria* VT1 and two strains of *V. filiformis* (ATCC 15551 and L-1401-7) are members of the *Betaproteobacteria* (Stahl et al., 1987) (Fig. BXII.β.84A). The 5S rRNA analyses carried out by Stahl et al. (1987) indicate that *V. filiformis* is related to *Rhodocyclus gelatinosus* in the *Chromobacterium* branch of the β-subdivision. Our revised analysis of the 5S rRNA sequence of *V. filiformis* places it in the *Rubrivivax–Leptothrix* sub-branch (Kalmbach et al., 1999) of the β1 subgroup of the *Proteobacteria*, most closely related to *Thiomonas cuprina* and *Leptothrix discophora* (Fig. BXII.β.84A).

Based on early 5S rRNA sequence data, *V. stercoraria* was originally placed at the base of the β1-β2 subgroups (Stahl et al., 1987) or within the β2 subgroup (Woese, 1987) of the β-*Proteobacteria*, and was considered to be somewhat related to *Neisseria* (Stahl et al., 1987). Later 16S rRNA analyses by Dewhirst et al. (1989) have confirmed the phylogenetic relatedness of *V. stercoraria* and *Neisseria* species (see Fig. BXII.β.84B), although those authors argued against including *V. stercoraria* in the *Neisseriaceae*. They pointed out that although the 16S rRNA of *V. stercoraria* has an average sequence identity of 93.7% with members of *Neisseria* (they average 95.5% within the genus), it should be separated from them because it lacks a key unique "*Neisseria* signature" sequence, it is a free-living organism, and it is oxidase negative (Dewhirst et al., 1989). It should be noted, however, that the current "Taxonomy" browser of the National Center for Biotechnology Information places the genus *Vitreoscilla* within the family *Neisseriaceae* (based on the 16S rRNA sequences of *V. stercoraria*).

Certain unclassified *Vitreoscilla* strains, morphologically similar to *V. stercoraria* (Costerton et al., 1961; Nichols et al., 1986; Strohl et al., 1986a), should now be considered as strains of *V. stercoraria*. Strain VT-1 is physiologically identical with *V. stercoraria* ATCC 15218, the type strain of the species (Strohl et al., 1986a). *Vitreoscilla* strains 389 and 390 (Costerton et al., 1961) have also been determined to be nearly 100% related by DNA hybridization to *Vitreoscilla stercoraria* strain ATCC 15218 (Nichols et al., 1986).

On the other hand, *Filibacter limicola*, an organism morphologically similar to *V. stercoraria*, has been shown to be completely unrelated to *V. stercoraria* (Clausen et al., 1985; Nichols et al., 1986).

Additional 16S rRNA sequences analyses and strain–strain nucleic acid hybridizations are clearly needed to place these organisms into their proper taxonomic locations with respect to related organisms. In particular, a serious taxonomic problem, which needs to be rectified, exists with the genus *Vitreoscilla*. *V. beggiatoides* was designated as the type species of the genus, based on the description of vitreoscillas as *Beggiatoa*-like organisms that do not deposit sulfur from hydrogen sulfide (Pringsheim, 1949c, 1951). Until the mid-1980s, however, the only well-characterized species of *Vitreoscilla* was *V. stercoraria*, since no other species from Pringsheim's collection had survived (Koch, 1964). Thus, strains did not exist for the type species of the genus until the description (Strohl et al., 1986a) of strains that fit the original description of *V. beggiatoides*. Now that the neotype strain of *V. beggiatoides* has been reasonably well characterized, and has been phylogenetically placed near, yet far enough to be distinct from, *Beggiatoa alba* in the class *Gammaproteobacteria*, the genus *Vitreoscilla* should be modified to contain only this single species. *V. stercoraria* is morphologically, ultrastructurally, nutritionally, physiologically, and ecologically very different from the other species (Strohl et al., 1986a) (Table BXII.β.102; Figs. BXII.β.80, BXII.β.81, BXII.β.82, BXII.β.83, and BXII.β.84). *V. stercoraria* and *V. filiformis*, both members of the class *Betaproteobacteria*, are not related to *V. beggiatoides*. Interestingly, *V. filiformis*, which more closely resembles *V. beggiatoides* morphologically, physiologically, and ecologically, is actually more closely related to *V. stercoraria*, although these two organisms are reasonably separated within the *Betaproteobacteria*. Because *V. beggiatoides* is the type species and the intent of the genus was to contain non-sulfur-depositing, beggiatoa-like organisms, it is proposed that *V. filiformis* and *V. stercoraria* be removed from the genus. *V. filiformis* and *V. stercoraria* should be taxonomically reclassified into separate genera more in line with their phylogenetic lineages. A fourth species, "*Vitreoscilla proteolytica*", was proposed in 1970 (Perschmann and Gräf, 1970) but the strain described possesses carotenoids (a characteristic never ascribed to *Vitreoscilla*), was never accepted as a new species of *Vitreoscilla*, and has not been further characterized phylogenetically; therefore it will not be considered here.

FURTHER READING

Dewhirst, F.E., B.J. Paster and P.L. Bright. 1989. *Chromobacterium, Eikenella, Kingella, Neisseria, Simonsiella*, and *Vitreoscilla* species comprise a major branch of the beta group *Proteobacteria* by 16S ribosomal ribonucleic acid sequence comparison: transfer of *Eikenella* and *Simonsiella* to the family *Neisseriaceae* (emend). Int. J. Syst. Bacteriol. *39*: 258–266.

Stahl, D.A., D.J. Lane, G.J. Olsen, D.J. Heller, T.M. Schmidt and N.R. Pace. 1987. Phylogenetic analysis of certain sulfide-oxidizing and related morphologically conspicuous bacteria by 5S ribosomal ribonucleic acid sequences. Int. J. Syst. Bacteriol. *37*: 116–122.

Strohl, W.R., T.M. Schmidt, N.H. Lawry, M.J. Mezzino and J.M. Larkin. 1986. Characterization of *Vitreoscilla beggiatoides* and *Vitreoscilla filiformis* sp. nov., nom. rev., and comparison with *Vitreoscilla stercoraria* and *Beggiatoa alba*. Int. J. Syst. Bacteriol. *36*: 302–313.

A
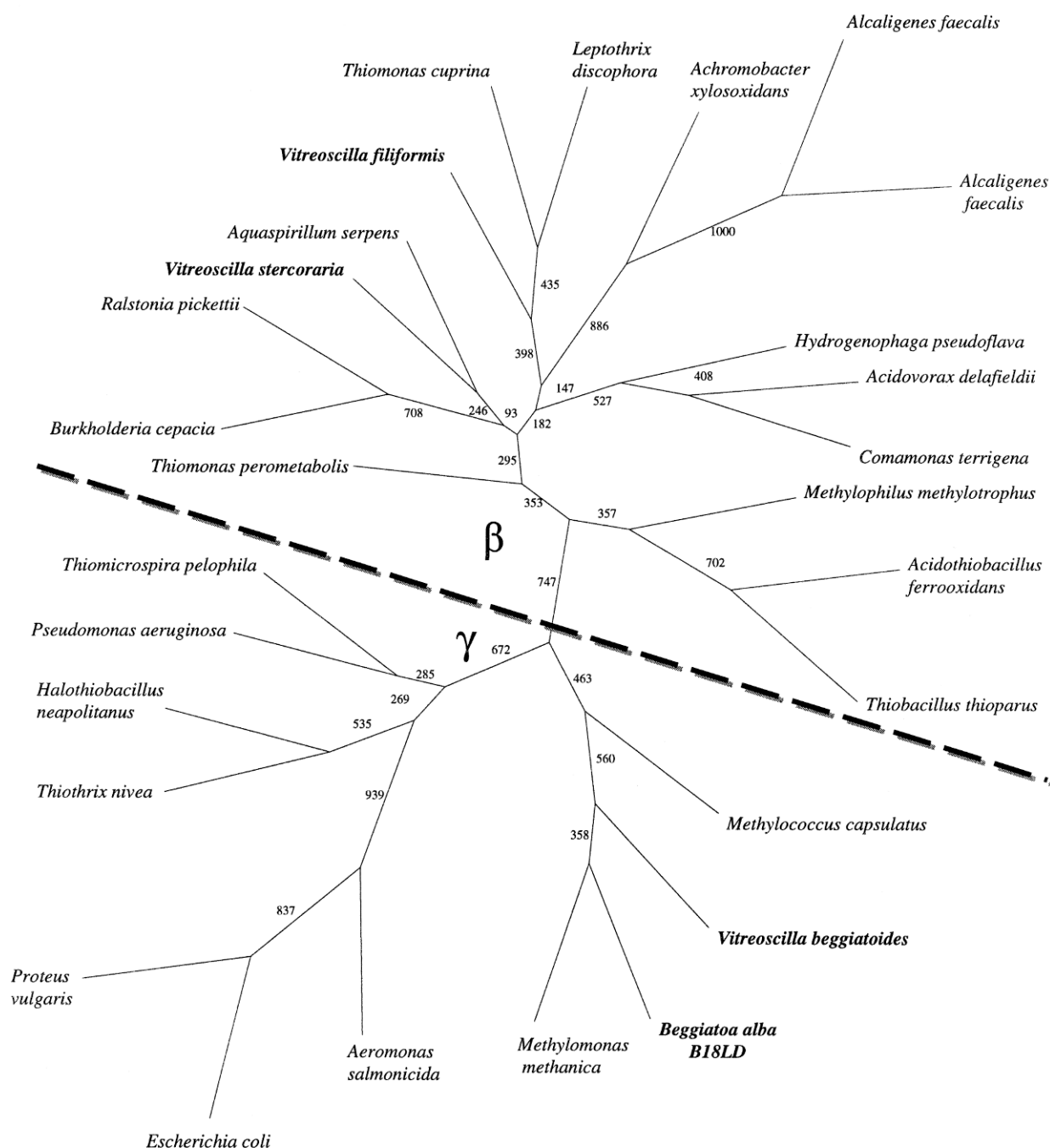
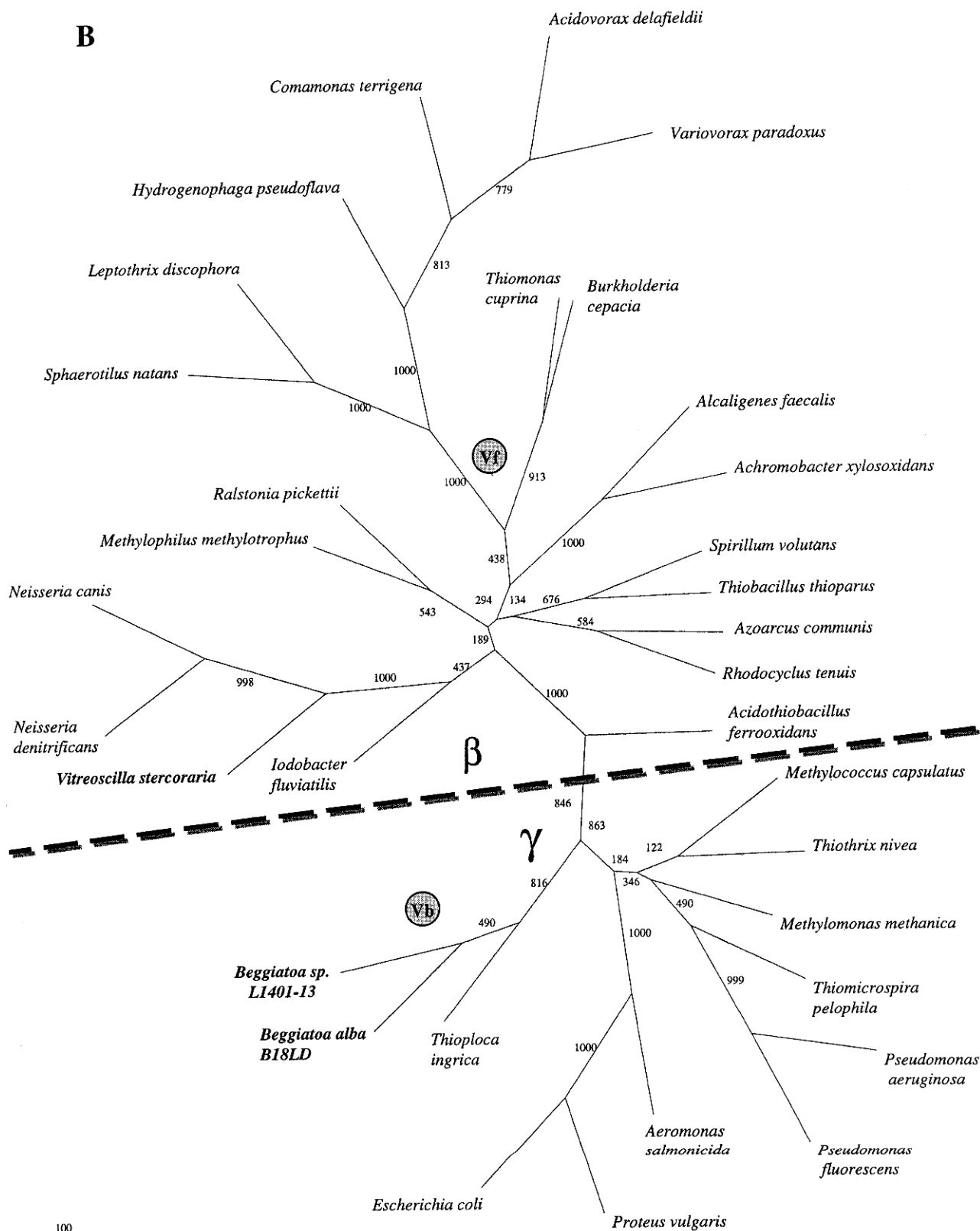**FIGURE BXII.β.84.** Unrooted distance trees of *Vitreoscilla* species, along with representative members of the classes *Betaproteobacteria* and *Gammaproteobacteria*. The sequences were aligned using the PILEUP program of the GCG Package, and the aligned sequences were compared using the DNADIST and NEIGHBOR programs of the PHYLIP (Felsenstein, 1993) software package. Bootstrap analyses were obtained from 1000 iterations. *A*, Phylogenetic analyses based on 5S rRNA sequences (in which all three species of *Vitreoscilla* are represented). *B*, Phylogenetic analyses based on 16S rRNA sequences, for which only *V. stercoraria* is the only *Vitreoscilla* species represented. The approximate positions of *V. filiformis* and *V. beggiatoides* are noted based on their nearest relatives with both 5S and 16S rRNA sequence alignments.

(continued)

B

Acidovorax delafieldii

Comamonas terrigena

Variovorax paradoxus

Hydrogenophaga pseudoflava

779

Leptothrix discophora

813

Thiomonas
cuprina

Burkholderia
cepacia

Sphaerotilus natans

1000

1000

Alcaligenes faecalis

Vf

Achromobacter xylosoxidans

1000

913

Ralstonia pickettii

Methylophilus methylotrophus

438

1000

Spirillum volutans

Neisseria canis

294   134   676

Thiobacillus thioparus

543

584

Azoarcus communis

189

Rhodocyclus tenuis

998

1000

437

1000

Acidothiobacillus
ferrooxidans

Neisseria
denitrificans

β

Vitreoscilla stercoraria

Iodobacter
fluviatilis

Methylococcus capsulatus

846

863

184   122

Thiothrix nivea

γ

346

816

Vb

490

490

Methylomonas methanica

1000

999

Thiomicrospira
pelophila

Beggiatoa sp.
L1401-13

Beggiatoa alba
B18LD

Thioploca
ingrica

1000

Pseudomonas
aeruginosa

Aeromonas
salmonicida

Pseudomonas
fluorescens

Escherichia coli

Proteus vulgaris

100

List of species of the genus Vitreoscilla

1. **Vitreoscilla beggiatoides** Pringsheim 1949c, 70,[AL] emend. Strohl, Schmidt, Lawry, Mezzino and Larkin 1986a, 311.

beg.gi.a.toi' des. M.L. fem. n. *Beggiatoa* a generic name; Gr. n. *idos* shape, form; M.L. adj. *beggiatoides Beggiatoa*-like.

TABLE BXII.β.101. Differentiation of the genus *Vitreoscilla* from other genera[a]

Characteristic	Vitreoscilla	Beggiatoa	Leptothrix	Neisseria	Thioploca
Colorless	+	+	+	±	+
Strains deposit sulfur	−	+	−	−	+
Ensheathed	−	−	+	−	+
Holdfast	−	−	±	−	−
Gliding motility	+	+	−	−	+
Flagellated	−	−	+	−	−
Filament formation	+	+	+	−	+
Individual cells observable within filaments	±	−	+	na	−
Cell division in two planes	−	−	−	+	−
Coccoid	−	−	−	+	−
Class:					
Betaproteobacteria	+		+	+	
Gammaproteobacteria	+	+			+

[a]Symbols: +, positive in >90% of strains; −, negative in >90% of strains; ±, positive in 10–90% of strains; na, not applicable.

The characteristics are as described for the genus and as listed in Table BXII.β.102, with the following additional features. Cells are cylindrical, 2.5–3.0 × 3–6 μm, and occur in filaments. The cells are not normally visible within the filaments. The filaments have a length of up to several hundred μm. Filaments lack visible septation, and the ends of the filaments are rounded. Necridia and hormogonia are produced. Motile by a gliding motility with speeds of ~3 μm/sec. Produce colonies of the circuitans type when grown on low-nutrient media and of the linguiformis type when grown in high-nutrient media. Trichomes often glide singly, away from other trichomes. Sulfur is not deposited when cells are grown on sulfide; however, sulfide at a concentration of ~1 mM is apparently not inhibitory. PHB is always present when cells are cultured on acetate-containing media and often comprises >50% of the cell dry weight. Polyphosphate bodies are often present.

Grows best at low nutrient concentrations. Acetate, ethanol, and some C_4 organic acids are utilized as sole carbon and energy sources. Ammonium and nitrate are used as nitrogen sources. Microaerophilic to aerobic. Gelatin, starch, and casein are not hydrolyzed. Catalase negative. Cytochrome oxidase positive. Growth occurs in 0.5% but not 1.0% NaCl. Sensitive to the antibiotics polymyxin B, neomycin, and furadantin/macrodantin; resistant to the antibiotics bacitracin and streptomycin. Found in sandy, but lightly sulfide-emanating, sediments of freshwater streams. Phylogenetic analysis places this species within the class *Gammaproteobacteria*, relatively close to *Beggiatoa alba*.

The mol% G + C of the DNA is: 42 (T_m, Bd).

Type strain: B23SS, ATCC 43189.

2. **Vitreoscilla filiformis** Strohl, Schmidt, Lawry, Mezzino and Larkin 1986a, 310[VP]

fi.li.for′ mis. L. n. *filum* a thread; L. n. *forma* shape; M.L. adj. *filiformis* thread-shaped.

The characteristics are as described for the genus and as listed in Table BXII.β.102, with the following additional features. Cells occur in flexible filaments with diameters of about 1.0–1.5 μm and, when cells are grown in liquid media, lengths of up to several hundred μm. Filament width may vary slightly. The cells are not clearly visible within the filaments, although slight indentations of the trichomes may sometimes be observed at septal regions between cells. The filament walls are continuous, and the ends of the filaments are rounded. Hormogonia are sometimes produced. Motile by gliding motility; gliding is relatively slow, at ~0.1–0.5 μm/sec. Produce linguiformis-type colonies. PHB and condensed phosphate deposits may be present.

Growth is best at low nutrient concentrations. Can use many 2-, 3-, 4-, and 6-carbon organic acids, several amino acids, ethanol, and glucose as sole carbon and energy sources. Organic and inorganic forms of nitrogen can be used as nitrogen sources. Nitrate is reduced to nitrite. Aerobic to microaerophilic. Gelatin and starch are weakly hydrolyzed. Casein is not hydrolyzed. Catalase negative, cytochrome oxidase positive. No growth occurs in the presence of 0.5% NaCl. Sensitive to the antibiotics bacitracin, streptomycin, furadantin/macrodantin, and polymyxin B. Found in freshwater sediments, usually in association with decaying matter. Phylogenetic analysis of *V. filiformis* places it into the *Betaproteobacteria*, with the closest relatives being *Thiomonas cuprina* and *Leptothrix*.

The mol% G + C of the DNA is: 59–63 (T_m).

Type strain: L1401-2, ATCC 43190.

3. **Vitreoscilla stercoraria** Pringsheim 1951, 136[AL] emend. Strohl, Schmidt, Lawry, Mezzino and Larkin 1986a, 312.

ster.co.ra′ ri.a. L. fem. adj. *stercoraria* pertaining to dung.

Cells usually occur as flexible chains with diameters of about 1.0 μm and, when grown in liquid media, lengths of up to about 100 μm. Deep constrictions separate the individual cells, yielding discontinuous filaments. Cells can occur singly, especially if grown at temperatures of ~22°C. The cells are sausage-shaped, 1.0 × 1.5–12.0 μm, and are connected in filaments by extracellular material. Division is by binary fission. PHB and condensed phosphate deposits may be present.

Grows luxuriantly on rich media such as 0.5% peptone broth. Growth occurs on Casamino acids plus acetate. Requires amino acids; arginine, tyrosine, tryptophan, and glutamine are required for good growth. Combinations of amino acids from the glutamate and aspartate families plus arginine are required minimally for growth. Cytochromes *a*, *c*, and the non-CO-binding *b* are not present. Contains unique bacterial hemoglobin. Aerobic. No growth microaerophilically or anaerobically. Catalase positive. Cytochrome oxidase negative. Gelatin, casein, and starch not hydrolyzed. Usually isolated from cow dung. Phylogenetic analysis of *V. stercoraria* places it into the class *Betaproteobacteria*, with the closest relatives being members of the genus, *Neisseria*.

The mol% G + C of the DNA is: 50–51 (Bd).

Type strain: SAG 1488-6, ATCC 15218, DSM 513.

GenBank accession number (16S rRNA): L06174.

TABLE BXII.β.102. Differential characteristics of the species of the genus *Vitreoscilla*[a]

Characteristic	V. beggiatoides	V. filiformis	V. stercoraria
Colony type	L, C	L	L
Filament type:[b]			
Continuous cell wall	+	+	−
Discontinuous cell wall	−	−	+
Filament diameter (μm)	2.5–3.0	1.0–1.5	1
Habitat:			
Freshwater sediments	+	+	−
Cow dung	−	−	+
Obligatory requirement for amino acid mixtures	−	−	+
Growth on:			
Nutrient agar	−	−	+
Acetate plus salts	+	+	−
Use as sole carbon and energy source:			
Glucose, citrate, lactate, glutamate	−	+	−
Succinate, acetate	+	+	−
Utilization of nitrate as sole nitrogen source	+	+	−
Nitrogen fixation	+	+	nd
Cytochromes:			
CO-binding types	+	+	+
c-type	+	+	−
Bacterial hemoglobin	nd	nd	+
Mol% G + C of DNA	42	59–63	50–51

[a]For symbols see standard definitions; L, linguiformis type colonies; C, circuitans type colonies (see Pringsheim, 1964 and Strohl and Larkin, 1978b for a description of these colony types); nd, not determined.

[b]Cells within filaments having continuous cell walls share the outer cell wall layers, with at most only shallow constrictions between cells noticeable (cf. Figs. BXII.β.80 and BXII.β.81). Strains having discontinuous filament cell walls consist of chains of individual cells held together by connective material (c.f. Figs. BXII.β.82 and BXII.β.83).

Genus XIII. **Vogesella** *Grimes, Woese, Macdonell and Colwell 1997, 25*[VP]

NOEL R. KRIEG

Vo.ges.el′ la. Ger. *Voges* proper name; M.L. *-ella* dim. ending; M.L. fem. n. *Vogesella* named after Otto Voges to honor his original isolation of *Bacillus indigoferus* on gelatin plates inoculated with tap water from the central water supply system in Kiel, Germany, in 1893.

Straight rods, 0.5 × 3.5 μm, mainly occurring singly but also in pairs and short chains. Occasional vibrioid rods may occur. Most strains form filamentous rods and long chains when grown on nitrogen-limiting agar. **Motile by means of a single polar flagellum.** Poly-β-hydroxybutyrate granules occur, especially under nitrogen limitation. **Colonies are a deep royal blue with a metallic copper-colored sheen at 36 to 48 h, due to production of indigoidine** ($C_{10}H_8N_4O_4$ or 5,5′-diamino-4,4′-dihydroxy-3,3′-diazadiphenoquinone-[2,2′]). **Oxidase and catalase positive. Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. All strains can carry out denitrification with production of gas. **Nonfermentative.** Citrate, several amino acids and peptides, and some monosaccharides are utilized as carbon sources. Starch, pectin, and sugars associated with pectin (i.e., arabinose and galactose) are not metabolized. Nonlipolytic. **Casein, gelatin, and esculin are not hydrolyzed. Indole positive.** Belong to the *Betaproteobacteria* and are closely related to the genus *Chromobacterium*. Occur in freshwater habitats and oxidation pond sediment.

The mol% G + C of the DNA is: 65.4–68.8.

Type species: **Vogesella indigofera** (Voges 1893) Grimes, Woese, Macdonell and Colwell 1997, 25 (*Pseudomonas indigofera* (Voges 1893) Migula 1900, 950; *Bacillus indigoferus* Voges 1893, 307.)

FURTHER DESCRIPTIVE INFORMATION

Colonies begin as translucent slightly yellowish colonies at early stages of growth (i.e., 16 to 20 h), then assume a faint bluish hue (24 h), and finally become a deep royal blue with a metallic copper-colored sheen (36 to 48 h). The pigment is localized within the cytoplasmic membrane (Kolar, 1974). Slightly acidic conditions (ca. pH 6.5) favor pigment production on plate count agar (PCA, Difco Laboratories, Detroit, MI). Excess pigment may occur in the colonies in the form of bluish crystals. When a colony is removed from an agar plate, a blue imprint or stain is left in the agar. Abundant blue pigmentation occurs on PCA, Thayer-Martin agar, chocolate agar, blood agar, *Pseudomonas* F agar, and especially chalk agar (Starr et al., 1960). Indigoidine production appears to be a stable trait of *Vogesella* strains.

Violacein (a violet pigment characteristic of *Chromobacterium* and *Janthinobacterium* species) and pyoverdin are not formed. All *Vogesella* strains exhibit a very weak fluorescence on chalk agar with short-wavelength UV illumination.

Growth occurs at 4°C and 37°C but not at 2°C and 40°C. Growth occurs at pH 6.2 and most strains grow at pH 8.6, but not at pH 4.0.

DNase is produced. The following tests are negative: methyl red; Voges–Proskauer; malonate; H_2S production; arginine, lysine, ornithine, and tryptophan decarboxylases; phenylalanine deaminase; urease; hydrolysis of esculin, *o*-nitrophenyl-β-D-galactopyranoside (ONPG), Tween 80, and corn oil.

All or most strains can use D-fructose, D-glucose, glycerol, ethanol, D-alanine, L-alanine, L-arginine, L-histidine, L-ornithine, L-proline, γ-aminobutyrate, fumarate, and putrescine as sole carbon sources. Propionate is not used.

Blue-pigmented bacteria appear to be a consistent feature of freshwater bacterial communities in terms of temporal and spatial distribution (Grimes et al., 1997).

ENRICHMENT AND ISOLATION PROCEDURES

Isolates can be selected from PCA plates that have been spread with water or sediment samples and incubated at 20 to 25°C for 48 h. Colonies are selected that have a blue pigmentation and a metallic copper-colored sheen.

MAINTENANCE PROCEDURES

Pure cultures can be maintained in deeps of $0.5 \times$ plate count broth (Difco) containing 0.3% agar (Difco) and stored at room temperature (ca. 20°C). Cultures maintained in this manner can remain viable for more than 10 years. Cultures can also be stored indefinitely under liquid nitrogen in tryptic soy broth (Difco) containing 5% glycerol.

DIFFERENTIATION OF THE GENUS *VOGESELLA* FROM OTHER GENERA

Arthrobacter spp., *Corynebacterium insidiosum*, and *Erwinia chrysanthemi* also form indigoidine. *Arthrobacter* and *Corynebacterium* are Gram positive, whereas *Vogesella* is Gram negative. Unlike *Vogesella*, *Erwinia* has peritrichous flagella, is oxidase negative, and can ferment sugars.

Chromobacterium species form violet-pigmented colonies that might be confused with those of *Vogesella*, although the pigment is violacein, not indigoidine. Unlike *Vogesella*, *Chromobacterium* is facultatively anaerobic, has a mainly fermentative attack on carbohydrates, is indole negative, digests casein, and does not denitrify.

Janthinobacterium species, which have an oxidative type of metabolism, produce violet-pigmented colonies due to formation of violacein. Unlike *Vogesella* strains, *Janthinobacterium* is indole negative, hydrolyzes esculin, does not grow at 37°C, and does not denitrify.

TAXONOMIC COMMENTS

A phylogenetic study of 13 new isolates by Grimes et al. (1997), in which both 5S rRNA and 16S rRNA analyses were employed, indicated that the isolates were closely related to each other and to strains *Pseudomonas indigofera* ATCC 19706[T] and *P. indigofera* ATCC 14036. In addition, both 5S rRNA and 16S rRNA analyses demonstrated that the new isolates and strains ATCC 19706[T] and ATCC 14036 were members of the *Betaproteobacteria*. Moreover, although these strains were closely related to *Chromobacterium violaceum* ATCC 12742[T], they were sufficiently distinct to warrant placement in a new genus, *Vogesella*.

List of species of the genus Vogesella

1. **Vogesella indigofera** (Voges 1893) Grimes, Woese, Macdonell and Colwell 1997, 25[VP] (*Pseudomonas indigofera* (Voges 1893) Migula 1900, 950; *Bacillus indigoferus* Voges 1893, 307.)

   in.di.go′ fe.ra. Fr. n. *indigo* the dye indigo [from India]; L. suff. *fer* from L. v. *fero* to bear; M.L. adj. *indigofera* bearing indigo.

The characteristics are as described for the genus.
The mol% G + C of the DNA is: 65.4–68.8 (T_m).
Type strain: ATCC 19706, CCUG 2873, CCUG 32860, CIP 103306, DSM 3303, LMG 6867.
GenBank accession number (16S rRNA): U45995.

Genus Incertae Sedis XIV. **Catenococcus** Sorokin 1994, 852[VP] (Effective publication: Sorokin 1992, 2291)

DIMITRY Y. SOROKIN

Caten.o.coc′ cus. L. n. *catena* chain; Gr. n. *coccus* berry; *Catenococcus* a chain of berries.

Cells **coccoid**, occurring mostly in **chains**. **Gram negative. Nonmotile.** Metabolism **respiratory** and **fermentative**; **facultative anaerobe.** Neutrophilic and mesophilic. **Obligately heterotrophic**; able to utilize some sugars, sugar alcohols, and organic acids as carbon and energy sources. **Oxidize thiosulfate, sulfide, and S⁰ incompletely to tetrathionate** during heterotrophic growth. Able to **reduce S⁰** and **ferric iron** in the presence of fermentable substrate under microaerobic and anaerobic conditions. Oxidase and catalase positive.

Isolated from sulfidic marine water.
The mol% G + C of the DNA is: 49.8 ± 0.5 (T_m).
Type species: **Catenococcus thiocycli** Sorokin 1994, 852 (*Catenococcus thiocyclus* (sic)) (Effective publication: Sorokin 1992, 2291.)

FURTHER DESCRIPTIVE INFORMATION

Cells grown in batch culture occur in long chains (Fig. BXII.β.85A and B), connected by slime discs. Cells grown in continuous cultures usually occur in pairs or in short chains. The cell wall has a structure that is typical of Gram-negative bacteria

(Fig. BXII.β.85C). Growth is NaCl-dependent and occurs over a range of 0.5–8% (w/v) NaCl (optimal, 2.5–3.5%). Cell wall stability demands high Mg^{2+} concentrations. The pH range for growth is 5.6–7.8 (optimal, 6.7–6.9). Growth above pH 7.5 and with low Mg^{2+} concentrations results in sphaeroplast formation and rapid cell lysis. The temperature range for growth is 10–35°C (optimal, 25–28°C).

Catenococcus grows best aerobically with starch and sugars (D-glucose, D-maltose, sucrose, D-fructose, L-arabinose, D-trehalose, and D-cellobiose) as carbon and energy sources, presumably because of slight acidification of the medium. All of these sugars also support anaerobic growth, except L-arabinose. The main products of glucose fermentation are formate and acetate. Aerobic growth also occurs with acetate, succinate, malate, fumarate, pyruvate, gluconate, citrate, D-lactate, malonate, 2-oxoglutarate, propionate, butyrate, ethanol, propanol, glycerol, D-mannitol, L-aspartate, L-glutamate, L-proline, L-cysteine, and L-histidine. Growth is vitamin independent. Tween-80 and gelatin are not hydrolyzed. Ammonium salts, nitrate, and nitrite serve as nitrogen sources. Nitrate is reduced to nitrite.
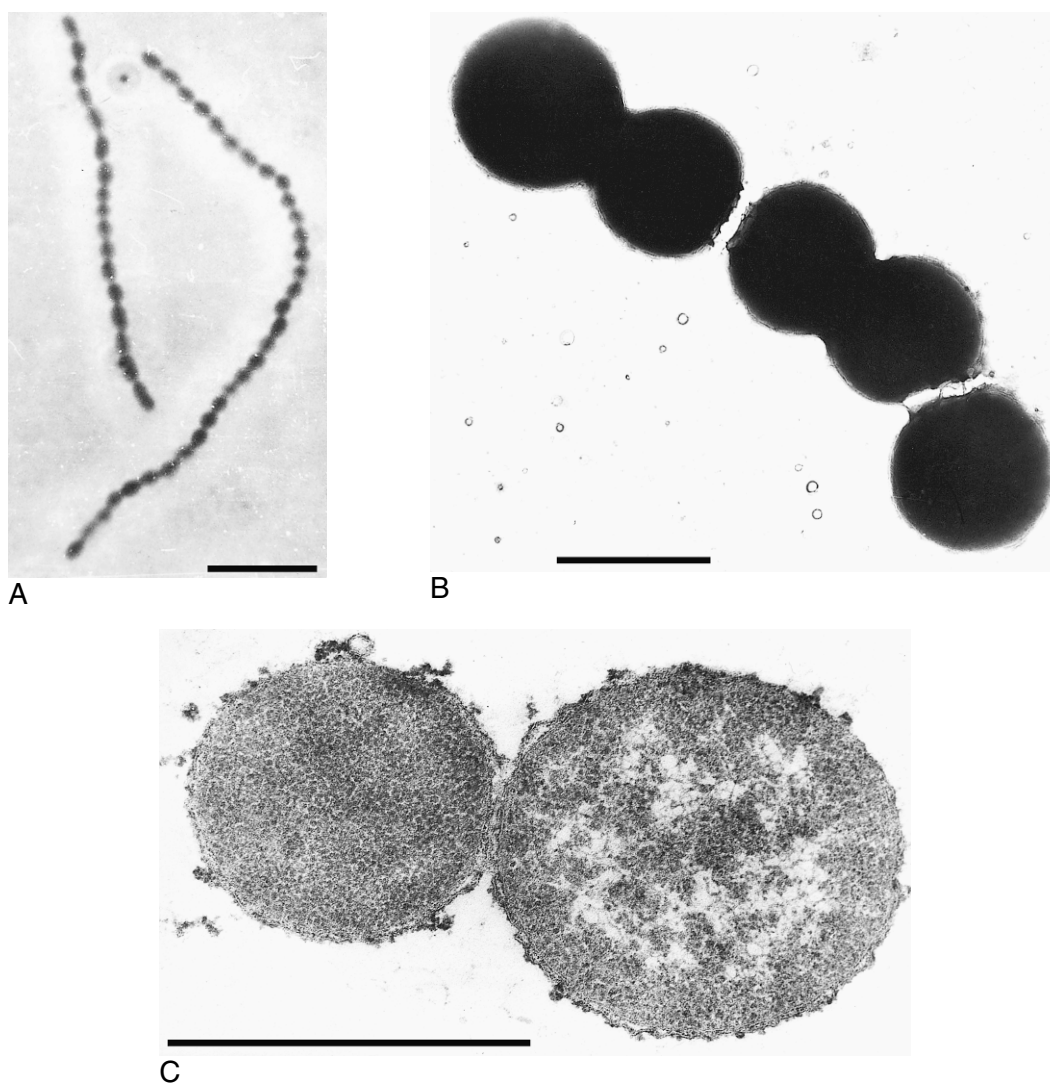
FIGURE BXII.β.85. Morphology of *Catenococcus thiocycli*. *(A)* phase-contrast photograph (Bar = 10 μm); *(B)* electron micrograph, total preparation stained with ammonium molybdate (Bar = 1 μm); *(C)* electron micrograph, thin section, stained with uranyl acetate + lead citrate (Bar = 1 μm).

Cultures oxidize thiosulfate to tetrathionate with high activity and sulfide to S^0 with much less activity. They are unable to grow autotrophically with reduced sulfur compounds, but can utilize metabolic energy released during thiosulfate oxidation to tetrathionate, as has been revealed in chemostat experiments (thiosulfate-dependent yield increase), and with washed cells (thiosulfate-dependent ATP synthesis). These data can be taken to imply the ability of *C. thiocycli* to grow chemolithoheterotrophically. Moreover, the bacterium is also capable of the reduction of S^0 to sulfide and of ferric iron to ferrous iron in the presence of glucose under either microaerobic or anaerobic conditions. However, these reactions do not support anaerobic respiration.

C. thiocycli is sensitive to chloramphenicol, streptomycin, tetracycline, erythromycin, ampicillin, kanamycin, colistin sulfate, nitrofurantoin, sulfafurazole, and penicillin G.

C. thiocycli has been isolated as a dominant tetrathionate-forming heterotroph from a near-shore volcanic thermal region (Papua, New Guinea) rich in sulfide and ferric iron. Its versatile metabolic potential suggests that such heterotrophs may play an important role in inorganic, as well as organic, cycling.

ENRICHMENT AND ISOLATION PROCEDURES

Catenococcus can be enriched, isolated, and successfully grown on a medium with the following composition (g/l): NaCl, 25; NH_4Cl, 0.5; potassium phosphate buffer pH 7, 2–5; $MgSO_4 \cdot 7H_2O$, 0.5–1; $CaCl_2 \cdot 2H_2O$, 0.1; yeast extract, 0.05; acetate or D-glucose, 10–20 mM; sodium thiosulfate, 20 mM; trace elements (Pfennig and Lippert, 1966), 1 ml/l.

MAINTENANCE PROCEDURES

Active cultures can be maintained in liquid culture or on agar slants at 4°C for 6 months without significant loss of viability. They survive lyophilization and may also be stored in liquid nitrogen.

DIFFERENTIATION OF THE GENUS *CATENOCOCCUS* FROM OTHER GENERA

C. thiocycli differs from the morphologically similar *Paracoccus* by its low mol% G + C and inability to grow autotrophically, from *Neisseria* by its fermentative potential, and from fermentative bac-

teria by its morphology. The main differences between *Cateno-coccus* and *Neisseria* are given in Table BXII.β.103.

TAXONOMIC COMMENTS

At the time this genus was described, phylogenetic analysis was not widespread. Therefore, until recently, the true relatives of this organism and its taxonomy remained unclear. Based on phenotypic characteristics, *Catenococcus* was placed in the family *Neisseriaceae* of the *Betaproteobacteria*, which includes mostly nonfermentative, often pathogenic, coccoid bacteria. Recently, partial

16S rDNA sequencing of *C. thiocycli* was performed which demonstrated that this bacterium is within the radiation of the *Vibrionaceae* of the *Gammaproteobacteria* and is closely related to *Listonella pelagia* (96% sequence identity). The fermentative capability of *C. thiocycli* is in agreement with this positioning, but strong morphological differences exist between *Catenococcus* and all other members of the *Vibrionaceae*.

FURTHER READING

Sorokin, D.Y., L.A. Robertson and J.G. Kuenen. 1996. Sulfur cycling in *Catenococcus thiocyclus*. FEMS Microbiol. Ecol. *19*: 117–125.

TABLE BXII.β.103. Comparison of *Catenococcus thiocycli* with the genus *Neisseria*

Characteristic	*C. thiocycli*	*Neisseria*
Morphology	Cocci in chains	Diplococci or cocci in short chains; one species rod-shaped
Division	One plane	Two planes
Acid production from sugars	+	D
Anaerobic growth with sugars	+	−
Anaerobic growth with nitrite	−	D
Mol% G + C	49.8	46.5–55.6
Habitat	Sulfidic seawater	Mammalian material

List of species of the genus Catenococcus

1. **Catenococcus thiocycli** Sorokin 1994, 852^{VP} (*Catenococcus thiocyclus* (sic)) (Effective publication: Sorokin 1992, 2291.) thi.o.cy′cli. Gr. n. *thios* sulfur; Gr. n. *cyclos* circle.

Characteristics are those of the genus. The type strain was isolated from sulfidic seawater in Papua New Guinea.
The mol% G + C of the DNA is: 49.8 ± 0.5 (T_m).
Type strain: TG 5-3, ATCC 51228, DSM 9165, LMD 92.12.

Genus Incertae Sedis XV. **Morococcus** *Long, Sly, Pham and Davis 1981, 300^{VP}*

LINDSAY I. SLY

Mo.ro.coc′cus. L. neut. n. *morum* mulberry; Gr. n. *coccus* a grain or berry; M.L. masc. n. *Morococcus* the mulberry coccus.

Colorless, **spherical organisms**, <1 μm in diameter, bound firmly together in tightly packed, **mulberry-like aggregates** of 10–20 cells, with **adjacent sides often flattened**. **Gram negative**. **Non-motile**. Endospores not formed. **Aerobic**. Nitrate is reduced. Catalase and cytochrome oxidase are produced. H_2S is produced. Acid is produced from carbohydrates. **Hemolytic**. Complex growth factors not required. Growth occurs between 23° and 42°C and between pH 5.5 and 9.0. Ecological niche unknown, but originally isolated from a human brain abscess.

The mol% G + C of the DNA is: 52.

Type species: **Morococcus cerebrosus** Long, Sly, Pham and Davis 1981, 300.

FURTHER DESCRIPTIVE INFORMATION

The description of the genus *Morococcus* is based on the characteristics of a single isolate designated as the type strain of the type species *Morococcus cerebrosus*. Thus, the diversity of the species characteristics is unknown. The type strain was isolated from a cerebellar abscess in a 54-year-old woman in 1971 in Australia. The following comments are based on the original description by Long et al. (1981). The type strain grows well on a variety of nutrient media including peptone yeast extract agar (PYEA) and chocolate agar. The addition of glucose enhances growth. Growth occurs on agar containing only peptone or yeast extract, but better growth occurs in media containing both components.

Growth is poor on vitamin-free Casamino acids medium, but improved when yeast extract is added.

On PYEA after 24 h, colonies are 1 mm in diameter and have a frosted-glass appearance under reflected light. The colonies remain intact when moved and are difficult to emulsify. Colonies grown on sucrose peptone agar stain black with Burke iodine stain and the cells are surrounded by a slime layer when stained with nigrosin.

Colonies appear to be constructed of masses of subunits 3–5 μm in diameter. Each subunit contains 10–20 individual cells approximately 0.8 μm in diameter (Fig. BXII.β.86). Individual cells, pairs, or tetrads are rarely observed. Morphological examination by light microscopy is difficult due to the cellular aggregates (Fig. BXII.β.87). Gram stains may appear Gram positive unless the ethanol decolorization step is extended for 20–40 s, and irregular staining is often observed.

Despite obvious morphological differences, the type strains of *Morococcus cerebrosus* and *Neisseria mucosa* show considerable biochemical similarity. However, the two type strains can be differentiated by several characteristics. *Neisseria mucosa* strongly reduces litmus milk and grows on MacConkey agar, but fails to produce acid reactions in Falkow decarboxylase tests. *Morococcus cerebrosus*, on the other hand, reduces litmus milk weakly, fails to grow on MacConkey agar, and gives a strong decarboxylase reaction for ornithine.
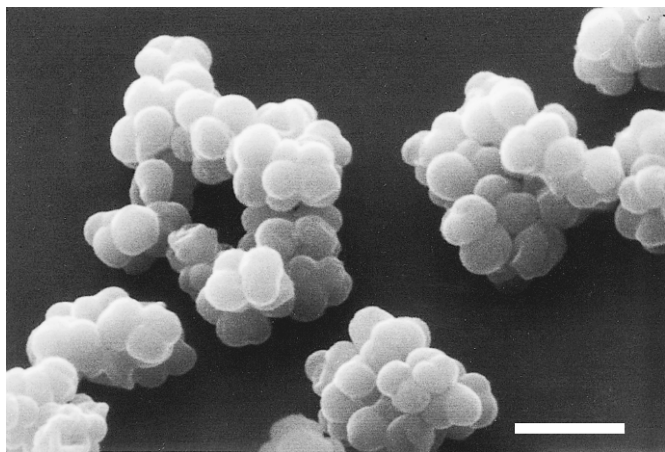
FIGURE BXII.β.86. Scanning electron micrograph of *Morococcus cerebrosus* ACM 858[T] showing mulberry-like cellular aggregates (Bar = 4 μm).
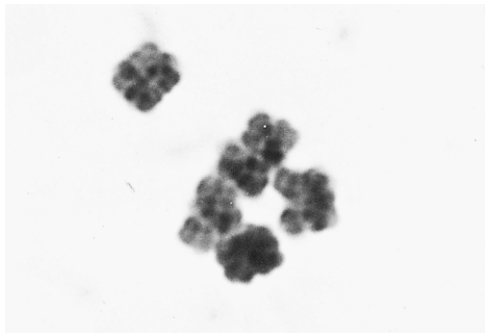

FIGURE BXII.β.87. Photomicrograph of methylene blue-stained cellular aggregates of *Morococcus cerebrosus* ACM 858[T] (× 3000 magnification).

Mice inoculated intraperitoneally with 0.5×10^6 colony-forming units of *Morococcus cerebrosus* became ruffled within 24 h but completely recovered by 48 h. Mice inoculated with 0.5×10^9 colony-forming units died within 24 h. Aggregates were observed microscopically in homogenized spleen samples from dead animals. Mice inoculated subcutaneously developed pyogenic lesions of varying sizes at the sites of inoculation and showed lymph node involvement that persisted for more than 7 d. The larger lesions contained free pus, in which the bacteria were observed by Gram staining and recovered by culture.

Antiserum raised against *Morococcus cerebrosus* in rabbits showed no affinity to *Neisseria mucosa* and when this antiserum was adsorbed with *Neisseria mucosa* it remained reactive to *Morococcus cerebrosus*, indicating the serological difference between these two type strains. These serological differences were confirmed by gel immunodiffusion and gel immunoelectrophoresis.

MAINTENANCE PROCEDURES

Morococcus cerebrosus may be grown on a variety of nutrient media and maintained routinely on peptone yeast extract agar (PYEA). The organism may be preserved by cryogenic storage in liquid nitrogen when suspended in PYE broth containing 10% glycerol, and by freeze drying in glucose peptone broth containing horse serum.

DIFFERENTIATION OF THE GENUS *MOROCOCCUS* FROM OTHER GENERA

The description of *Morococcus* supports its membership of the family *Neisseriaceae*. The closest phenotypic relatives are *Neisseria mucosa* and *Neisseria macacae* (Long et al. 1981; Barrett and Sneath 1994; Ben Dekhill, Stackebrandt and Sly, unpublished data). The cellular aggregates formed by *Morococcus* most clearly differentiate the genus from other members of the *Neisseriaceae*. Barrett and Sneath (1994) confirmed the unique mulberry-like aggregates of *Morococcus cerebrosus* and concluded that numerical analysis clustering supported the inclusion of the genus *Morococcus* within the *Neisseriaceae*. However, unpublished phylogenetic evidence (see Taxonomic Comments below) supports the inclusion of *Morococcus cerebrosus* in the genus *Neisseria*, a taxonomic change that would require emendation of the genus *Neisseria* to accommodate an aggregate forming species. The characteristics that differentiate the genus *Morococcus* from related Gram-negative genera with coccoid morphology are shown in Table BXII.β.104.

TAXONOMIC COMMENTS

In the original description of *Morococcus cerebrosus*, Long et al. (1981) made considerable efforts to distinguish *Morococcus cerebrosus* from *Neisseria mucosa* and concluded that *Morococcus cerebrosus* could be sufficiently differentiated from *Neisseria mucosa* based on physiological, cultural, serological, and morphological characteristics to warrant its description as a separate species in a new genus. Barrett and Sneath (1994) made an extensive numerical phenotypic study of *Neisseria* including *Morococcus cerebrosus*. *M. cerebrosus* was placed alone on a separate branch as a satellite of phenon 24 that contained strains from dental plaque

TABLE BXII.β.104. Differentiation of the genus *Morococcus* from related Gram-negative genera with coccoid morphology[a]

Characteristic[b]	*Morococcus cerebrosus*	*Neisseria*	*Moraxella* (*Branhamella*)
Cellular morphology:			
Cocci	+	+	+
Rods	−	+[c]	−
Cell aggregates	+	−	−
Acid from glucose	+	D	−
Nitrate reduced	+	D	D
Hemolysis of blood cells	+	D	D
Mol% G + C of DNA	52	46–56	40–47

[a]Symbols: +, present in all species; −, absent in all species; (+), weak reaction; d, 11–89% of strains are positive; D, variable reaction in different species.

[b]Data from Bøvre (1984a, b), Vedros (1984).

[c]*Neisseria elongata* only.

from gorilla and cows. The neighboring cluster (phenon 25) contained one of the replicates of the type strain of *Neisseria mucosa*, although the authors regarded this relationship as unusual. The other replicate was placed in phenon 9 together with one replicate of the type strain of *Neisseria macacae*. The other replicate of *N. macacae* was placed as a satellite of phenons 9–12.

Phylogenetically, *Morococcus cerebrosus* forms a clade with *Neisseria mucosa* and *Neisseria macacae* based on 16S rRNA gene sequence similarity (Ben Dekhill, Stackebrandt, and Sly, unpublished data). *Morococcus cerebrosus* should therefore be considered a species of the genus *Neisseria* but its relationship with *Neisseria mucosa* and *Neisseria macacae* requires further investigation. In a recent study of the phylogeny of the genus *Neisseria*, Harmsen et al. (2001) showed that *Neisseria mucosa* and *Neisseria macacae* had identical 16S rDNA (*E. coli* positions 54–510) and 23S rDNA (*E. coli* position 1400–1600) sequences. Taxonomic change at this time is considered premature until further strains of *Morococcus cerebrosus* are obtained, if possible, and their relationship to *N. mucosa* and *N. macacae* is determined. However, it appears that in the 30 years since the first isolation of *M. cerebrosus* no further isolates have been reported. Aggregate formation as observed in *M. cerebrosus* is inconsistent with the description of the genus *Neisseria* and the description would require emendation to include *M. cerebrosus*.

List of species of the genus Morococcus

1. **Morococcus cerebrosus** Long, Sly, Pham and Davis 1981, 300[VP]

   ce.re.bro′ sus. L. adj. *cerebrosus* pertaining to the brain, the original source of isolation of this organism.

   Colorless, spherical organisms, less than 1 μm in diameter, bound firmly together in tightly packed, mulberry-like aggregates of 10–20 cells, with adjacent sides often flattened. Gram negative. Nonmotile. Endospores not formed. Poly-β-hydroxybutyrate not produced. Capsules are produced in sucrose-containing media.

   Growth occurs on vitamin-free Casamino acids, peptone, yeast extract, and chocolate agars. No growth occurs on Sabouraud dextrose agar, Czapek Dox agar, or peptone yeast extract agar (PYEA) containing 40% bile, 0.1% potassium tellurite, or 10% NaCl, or in glucose ammonium sulfate broth. After 24 h colonies on PYEA are convex or umbonate, entire, matt, buff colored, 1 mm in diameter, and difficult to emulsify. Colonies on sucrose agar give a black reaction with iodine. Granular growth occurs in peptone yeast extract broth. Growth occurs between 23° and 42°C, and between pH 5.5 and 9.0.

   Aerobic, but may give weak acid reactions in both tubes of the Hugh and Leifson test. Nitrate is reduced to nitrite and gas but the reaction may be variable. Litmus milk is reduced. Hemolyses horse erythrocytes. H_2S is produced from cysteine. Deoxyribonuclease, ornithine decarboxylase, catalase, cytochrome oxidase, and methyl red positive. Acid is produced from glucose, fructose, sucrose, and maltose, but not from arabinose, ribose, xylose, rhamnose, galactose, mannose, sorbose, salicin, cellobiose, lactose, melibiose, trehalose, melezitose, raffinose, dextrin, inulin, starch, ethanol, glycerol, erythritol, adonitol, arabinol, dulcitol, mannitol, sorbitol, or inositol. Citrate and malonate are not oxidized. Does not hydrolyze starch, esculin, gelatin, casein, or Tween 80. Lecithinase, phosphatase, phenylalanine deaminase, and urease are not produced. Arginine, glutamic acid, and lysine decarboxylases are not produced. Indole is not produced. Serum liquefaction is negative.

   Susceptible to the following antibiotic disks: kanamycin (30 μg), gentamicin (10 μg), streptomycin (10 μg), penicillin G (10 U), ampicillin (2 μg), erythromycin (15 μg), nalidixic acid (30 μg), chloramphenicol (10 μg), and tetracycline (10 μg). Resistant to cloxacillin (5 μg) and methicillin (5 μg).

   Antigenically unrelated to *Neisseria mucosa* ACM 1903[T] (ATCC 19696[T]). Pathogenic to mice. Ecological niche unknown, but originally isolated from a human brain abscess in Australia.

   The mol% G + C of the DNA is: 52 (T_m).

   Type strain: ACM 858, ATCC 33486, NCTC 11393, UQM 858.

Order V. **Nitrosomonadales** *ord. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Ni.tro.so.mo.na.da′ les. M.L. fem. n. *Nitrosomonas* type genus of the order; *-ales* ending to denote order; M.L. fem. n. *Nitrosomonadales* the *Nitrosomonas* order.

The order *Nitrosomonadales* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the order contains the families *Nitrosomonadaceae*, *Gallionellaceae*, and *Spirillaceae*.

Order is morphologically, metabolically, and ecologically diverse. Includes organisms having spiral-shaped, stalked, coccal, rod-shaped, and pleomorphic cells. Includes organisms that can grow chemolithotrophically, mixotrophically, and chemoorganotrophically. Found in a variety of habitats.

Type genus: **Nitrosomonas** Winogradsky 1892, 127 (Nom. Cons. Opin. 23 Jud. Comm. 1958, 169.)

Family I. **Nitrosomonadaceae** *fam. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Ni.tro.so.mo.na.da′ce.ae. M.L. fem. n. *Nitrosomonas* type genus of the family; *-aceae* ending
to denote family; M.L. fem. pl. n. *Nitrosomonadaceae* the *Nitrosomonas* family.

The family *Nitrosomonadaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the family contains the genera *Nitrosomonas* (type genus), *Nitrosolobus*, and *Nitrosospira*.

Lithoautotrophic bacteria that oxidize ammonia.

Type genus: **Nitrosomonas** Winogradsky 1892, 127 (Nom. Cons. Opin. 23 Jud. Comm. 1958, 169.)

Genus I. **Nitrosomonas** *Winogradsky 1892, 127[AL] (Nom. Cons. Opin. 23 Jud. Comm. 1958, 169)*

HANS-PETER KOOPS AND ANDREAS POMMERENING-RÖSER

Ni.tro.so.mo′ nas. M.L. adj. *nitrosus* nitrous; Gr. fem. n. *monas* a unit, monad; M.L. fem. n. *Nitrosomonas* nitrite monad, i.e., the monad producing nitrite.

Spherical, ellipsoidal, or rod-shaped cells (Figs. BXII.β.88a, BXII.β.89a, BXII.β.90a, and BXII.β.91a). Strains belonging to the same species are generally very similar in the shape and size of their cells. Cells occur singly or occasionally in short chains. Depending on the growth conditions, cells are **free-living or embedded in slimy aggregates**. Gram-negative cell wall, but **some** marine representatives show an additional outer cell-wall layer composed of subunits arranged in a macromolecular array (Watson and Remsen, 1969). Motile cells possess **polar flagella**. **Intracytoplasmic membranes are arranged as flattened vesicles, primarily in the peripheral cytoplasm** (Figs. BXII.β.88b, BXII.β.89b, BXII.β.90b, and BXII.β.91b). **Carboxysomes** (Fig. BXII.β.92) are
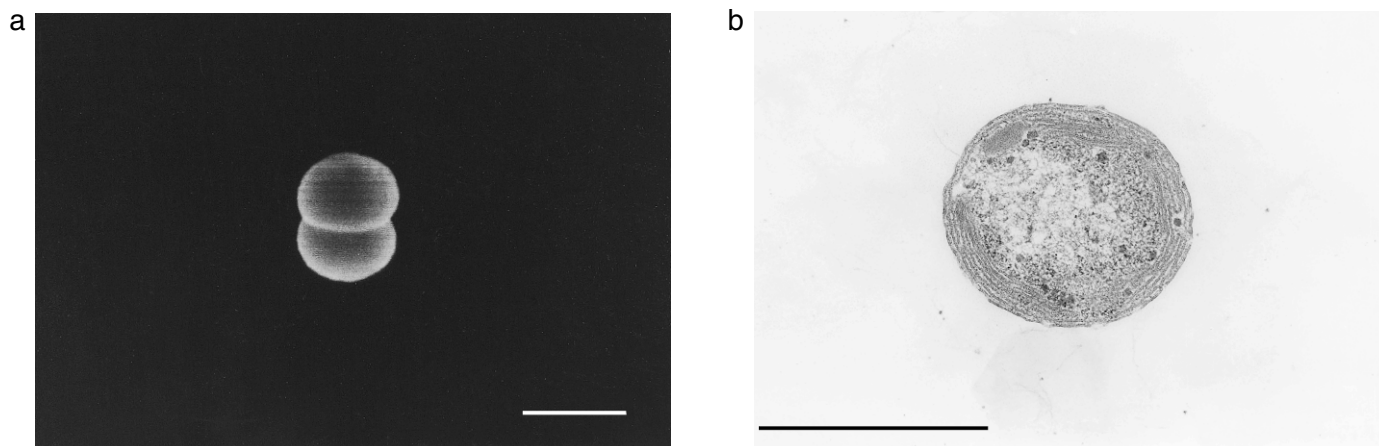
FIGURE BXII.β.88. (*a*) Scanning electron micrograph and (*b*) electron micrograph of a thin section of cells of *Nitrosomonas mobilis* (*"Nitrosococcus mobilis"*). Bars = 1 µm.
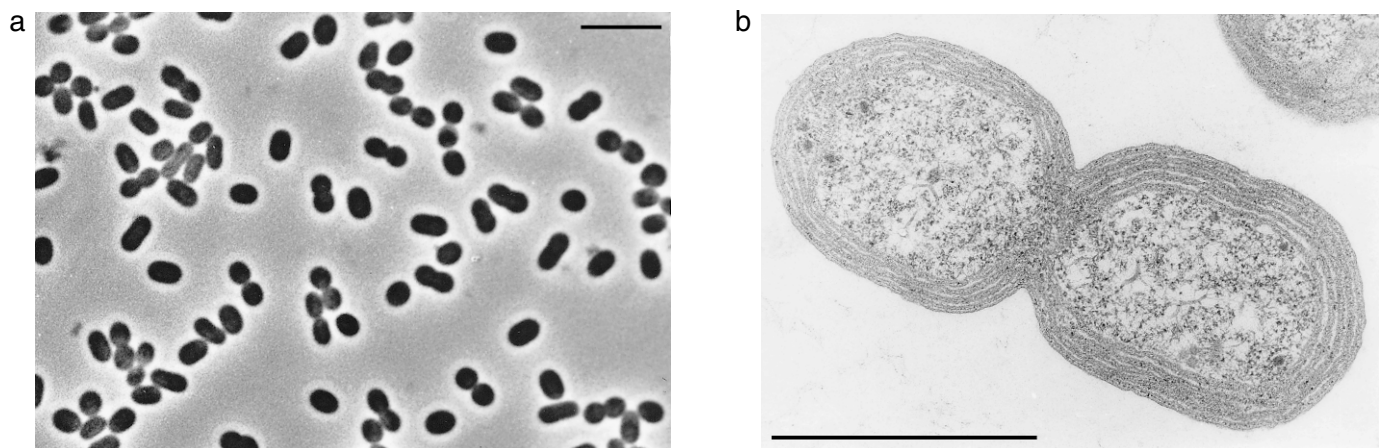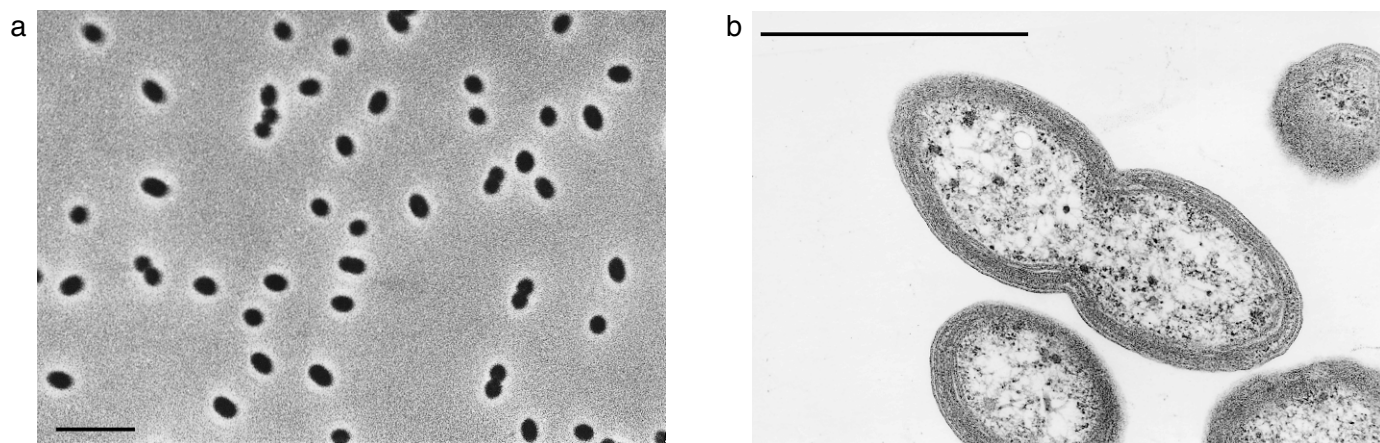
FIGURE BXII.β.89. (*a*) Phase contrast photomicrograph and (*b*) electron micrograph of a thin section of cells of *Nitrosomonas communis*. Bars = 5 µm (*a*) and 1 µm (*b*).

present in some species. **Urea** can be used as ammonia source by many, but not all, species. The K_s values for oxidation of NH_3 range between 0.6 and 158 µM. Most, if not all, species are predominantly or exclusively present in special environments.

The mol% G + C of the DNA is: 45–54 (T_m).

Type species: **Nitrosomonas europaea** Winogradsky 1892, 127, emend. mut. char. Watson 1971b, 266.

Eleven species of the genus *Nitrosomonas* are described. Together with other cultured, but undescribed, species of the genus and the reclassified *"Nitrosococcus mobilis"* (proposed here as *Nitrosomonas mobilis* comb. nov.), they form one of the two clusters of ammonia oxidizers within the *Betaproteobacteria*. Seven distinct phylogenetic lineages can be distinguished within this cluster, reflecting ecophysiologically distinct groupings (Table BXII.β.105).

DIFFERENTIATION OF THE SPECIES OF THE GENUS *NITROSOMONAS*

Table BXII.β.105 lists characteristic features useful for phenotypic differentiation of the cultured species of *Nitrosomonas*.

List of species of the genus Nitrosomonas

1. **Nitrosomonas europaea** Winogradsky 1892, 127[AL] emend. mut. char. Watson 1971b, 266.*

   eu.ro.pae′ a. Gr. adj. *europaeus* of Europe, European.

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*Editorial Note: Readers are advised that although the species of *Nitrosomonas* listed here appeared on Validation List No. 83 (IJSEM 2001, *51:* 1945) type material was not deposited in two public service collections as required by Rule 27 and 30 of the Bacteriological Code as amended in 1999. As such, these names may be illegitimate and invalid, and their usage may be called into question until this matter is satisfactorily addressed.

Cells are rod shaped, with rounded or pointed ends, $0.8–1.1 \times 1.0–1.7$ µm, generally occurring as single cells. Gram-negative cell envelope. Motility is not observed. Carboxysomes are not present. Urease negative. At pH ~7.8, cultures tolerate ammonium salt concentrations of up to 400 mM. The K_s values of NH_3 oxidation range between 30 and 56 µM. No obligate salt requirement, but cultures exhibit salt tolerance of up to 500 mM NaCl. Most common in sewage disposal plants and in eutrophic waters, but occasionally found in soils.

FIGURE BXII.β.90. (*a*) Phase contrast photomicrograph and (*b*) electron micrograph of a thin section of cells of *Nitrosomonas europaea*. Bars = 5 µm (*a*) and 1 µm (*b*).
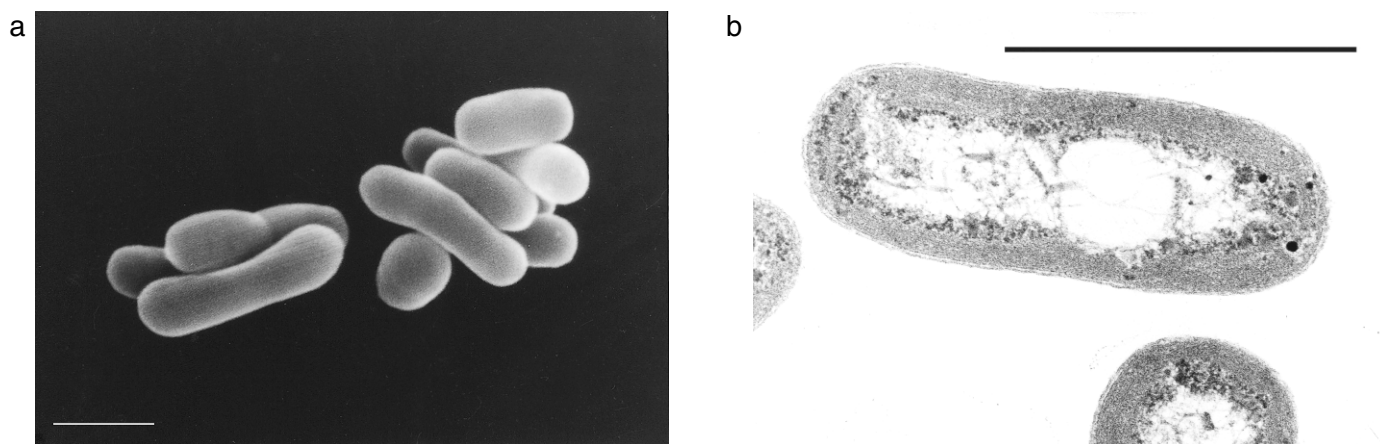

FIGURE BXII.β.91. (*a*) Scanning electron micrograph and (*b*) electron micrograph of a thin section of cells of *Nitrosomonas marina*. Bars = 1 µm.

FIGURE BXII.β.92. Electron micrograph of a thin section of a cell of *Nitrosomonas eutropha* showing carboxysomes (*arrows*). Bar = 1 μm.

The mol% G + C of the DNA is: 50.6–51.4 (T_m).
Type strain: ATTC 25978.
GenBank accession number (16S rRNA): M96399.

2. **Nitrosomonas aestuarii** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1697.*
ae.stu.a′ri.i. L. n. *aestus* tides; M.L. gen. n. *aestuarii* of the estuary.

Cells are rod shaped with rounded ends, 1.0–1.3 × 1.4–2.0 μm. Gram-negative cell wall. Additional layers, as found in *N. marina*, are not observed. Nonmotile. No carboxysomes. Cells can use urea as an ammonia source. The maximal ammonium salt tolerance is about 300 mM at pH 7.8. Cultures have an obligate salt requirement, with optimum growth at NaCl concentrations around 300 mM; 700 mM NaCl is tolerated. Isolated from marine environments, chiefly coastal waters.

Editorial Note: Readers are advised that although the species of *Nitrosomonas* listed here appeared on Validation List No. 83 (IJSEM 2001, *51:* 1945) type material was not deposited in two public service collections as required by Rule 27 and 30 of the Bacteriological Code as amended in 1999. As such, these names may be illegitimate and invalid, and their usage may be called into question until this matter is satisfactorily addressed.

The mol% G + C of the DNA is: 45.7–46.3 (T_m).
Type strain: Nm 36.
GenBank accession number (16S rRNA): AJ298734.

3. **Nitrosomonas communis** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1697.
com.mu′ nis. L. adj. *communis* common.

Cells are short rods or ellipsoidal with rounded ends, 1.0–1.4 × 1.7–2.2 μm. Gram-negative cell wall. Motility is not observed. No carboxysomes. Urea not used as an ammonia source. Ammonium salts tolerance is up to 200 mM at pH 7.8. The K_s value of NH$_3$ oxidation is 18–19 μM. No salt requirement; NaCl is tolerated up to 300 mM. Distributed mainly in agriculturally treated neutral soils.
The mol% G + C of the DNA is: 45.6–46.0 (T_m).
Deposited strain: Nm 2.
GenBank accession number (16S rRNA): AJ298732, Z46981.
Additional Remarks: At least two species, *Nitrosomonas* spp. I and II (Table BXII.β.105), exist that are physiologically not distinguishable from *N. communis* and are closely related to this species.

4. **Nitrosomonas eutropha** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1697.
eu.tro′ pha. Gr. pref. *eu-* good; *trophos* one who feeds; M.L. fem. n. *eutropha* good nutrition.

Pleomorphic cells, rod to pear shaped, with one or both ends pointed, 1.0–1.3 × 1.6–2.3 μm, occasionally in short chains. Gram-negative cell wall. Most strains are motile. Carboxysomes are present. Cells are urease negative. At pH ~7.8, cultures tolerate concentrations of ammonium salts of up to 600 mM. The K_s value of ammonia oxidation is 35–36 μM. No salt requirement, but up to 500 mM NaCl is tolerated. Commonly abundant in sewage disposal plants and in eutrophic waters. Occasionally found in soils.
The mol% G + C of the DNA is: 47.9–48.5 (T_m).
Type strain: C-91, Nm 57.
GenBank accession number (16S rRNA): AJ298739, AYl23795, M96402.

5. **Nitrosomonas halophila** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1698.*
hal.o′phi.la. Gr. n. *halos* salt; Gr. adj. *halophila* salt loving.

TABLE BXII.β.105. Differentiation of the species of the genus *Nitrosomonas*

Characteristic	*N. europaea*	*N. aestuarii*	*N. communis*	*N. eutropha*	*N. halophila*	*N. marina*	*N. mobilis*	*N. nitrosa*	*N. oligotropha*	*N. ureae*	"*N. cryotolerans*"	*Nitrosomonas* sp. I	*Nitrosomonas* sp. II	*Nitrosomonas* sp. III
Phylogenetic linage	1	5	4a	1	1	5	2	4b	3	3	6	4a	4a	5
Mol% G + C content of DNA	51.0	45.8	45.8	48.2	53.8	47.7	49.3	47.9	49.5	45.8	45.8	45.8	45.8	47.7
Salt requirement	−	+	−	−	+	+	+	−	−	−	+	−	−	+
Limit of NaCl tolerans (mM)	500	700	300	500	1000	800	600	200	200	300	600	300	300	800
K_s-value of ammonia oxidation (μM)	36		19.2	36				46	3.6	2.4		14.4	43	
Limit of NH$_4$Cl tolerance at pH 7.8 (mM)	400	300	200	500	400	200	300	100	50	100	400	200	200	200
Use of urea	−	+	−	−	−	+	−	+	+	+	+	−	−	+
Carboxysomes	−	−	−	+	+	−	−	+	−	−	−	−	−	+

Cells 1.1–1.5 × 1.5–2.2 µm, with a Gram-negative cell wall. Motility has not been observed. Carboxysomes are present. Urease negative. At pH ~7.8, cultures tolerate ammonium salts of up to 400 mM. Moderately halophilic; optimal NaCl concentration around 250 mM; tolerate up to 1000 mM NaCl. Strains were isolated from a brackish water environment (North Sea) and from soda lakes.

The mol% G + C of the DNA is: 53.8 (T_m).

Type strain: Nm 1.

GenBank accession number (16S rRNA): AJ298731, Z46987.

6. **Nitrosomonas marina** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1697.*

ma.ri′na. L. fem. adj. *marina* of the sea, marine.

Cells are generally slender rods with rounded ends, 0.7–0.9 × 1.4–2.3 µm. Gram-negative cell wall, but with an additional layer composed of subunits arranged in a macromolecular array. Motility is not observed. No carboxysomes. Cultures have an obligate salt requirement; the optimal NaCl concentration is around 400 mM; 800 mM NaCl is tolerated. Ammonium salts are tolerated at up to 200 mM at pH 7.8. Urease positive. Commonly distributed in marine environments.

The mol% G + C of the DNA is: 47.4–48.0 (T_m).

Type strain: Nm 22

GenBank accession number (16S rRNA): Z46990.

Additional Remarks: At least one further species, *Nitrosomonas* sp. III, exists (Table BXII.β.105) that is physiologically indistinguishable from *N. marina*, but cells are short rods and possess carboxysomes.

7. **Nitrosomonas mobilis** *comb. nov.* (*"Nitrosococcus mobilis"* Koops, Harms and Wehrmann 1976, 281).*

mo′bi.lis. L. adj. *mobilis* movable.

Cells generally spherical, 1.5–1.7 µm in diameter. However, some strains are rod shaped, 1.5–1.7 × 1.7–2.5 µm. Cells occur singly, in pairs, and occasionally as short chains. Gram-negative cell wall. Motile cells have a tuft of 1–22 flagella about 12 nm wide and 3–5 µm long. No carboxysomes. Cells are moderately halophilic, with an optimum NaCl concentration of about 100 mM. NaCl is tolerated at concentrations up to 600 mM. At pH 7.8, ammonium compounds are tolerated up to 300 mM. Urease negative. Strains have been isolated from brackish water environments and sewage disposal plants.

The mol% G + C of the DNA is: 49.3 (T_m).

Type strain: Nc 2.

GenBank accession number (16S rRNA): AF287297, AJ298728, M96403.

8. **Nitrosomonas nitrosa** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1697.*

ni.tro′sa. M.L. fem adj. *nitrosa* nitrous.

Spheres or short rods with rounded ends, 1.3–1.5 × 1.4–2.2 µm. Gram-negative cell wall. Motility not observed. Carboxysomes are present. Urea can serve as an ammonia source. Maximum tolerance of ammonium salts at pH 7.8 is 100 mM. The K_s value of NH_3 oxidation is 45–46 µM. No salt requirement; 200 mM NaCl is tolerated. Isolates originate from industrial sewage disposal plants, ponds, and occasionally rivers and soils.

The mol% G + C of the DNA is: 47.9 (T_m).

Type strain: Nm 90.

GenBank accession number (16S rRNA): AJ298740.

9. **Nitrosomonas oligotropha** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1698.*

o.li.go.tro′pha. Gr. adj. *oligos* little; Gr. n. *trophos* one who feeds; M.L. fem. n. *oligotropha* little nutrition.

Cells are rod shaped or spherical, 0.8–1.2 × 1.1–2.4 µm in size, with rounded ends. Gram-negative cell wall. Motility not observed. Slimy cell aggregates generally occur in cultures after exponential growth has ceased. No carboxysomes. Urease positive. Sensitive to increasing concentrations (>50 mM at pH 7.8) of ammonium salts. The K_s value of NH_3 oxidation is 2.4–4.2 µM. No salt requirement. NaCl is tolerated at up to 200 mM. Most strains have been isolated from oligotrophic freshwater environments.

The mol% G + C of the DNA is: 49.5–50 (T_m).

Type strain: Nm 45.

GenBank accession number (16S rRNA): AJ298736.

10. **Nitrosomonas ureae** Koops, Böttcher, Möller, Pommerening-Röser and Stehr 1991, 1698.*

u′re.ae. M.L. n. *urea* urea; M.L. gen. n. *ureae* of urea.

Cells are rod shaped, 0.9–1.1 × 1.5–2.5 µm, with rounded ends. Gram-negative cell wall. Motility is not observed. No carboxysomes. Urease positive. Ammonium salts are tolerated at up to 100 mM at pH 7.8. The K_s value of NH_3 oxidation is 1.9–3.6 µM. No salt requirement. NaCl is tolerated at up to 300 mM. Mainly found in oligotrophic fresh water environments and occasionally in soils.

The mol% G + C of the DNA is: 45.6–46.0 (T_m).

Type strain: Nm 10.

GenBank accession number (16S rRNA): AJ298730.

11. **"Nitrosomonas cryotolerans"** Jones, Morita, Koops and Watson 1988, 1122.

cry.o.to.le′rans. Gr. n. *cryos* cold, frost; L. pres. part. *tolerans* tolerating; M.L. part. adj. *cryotolerans* tolerating cold.

Cells are rod shaped with rounded ends, 1.2–2.2 × 2.0–4.0 µm. Gram-negative cell wall, but with an additional outer, electron-dense layer. Besides the flattened peripheral intracytoplasmic membranes that are typical of *Nitrosomonas*, membranes may intrude deep into the cytoplasm, forming what appear to be membrane-bound vesicles. Motility not observed. No carboxysomes. Urease positive. Cultures tolerate ammonium salts at up to 400 mM at pH 7.8. Cells have an obligate salt requirement, with optimal growth around 300 mM NaCl; 600 mM NaCl is tolerated. Cultures can grow at temperatures down to −5°C. The only existing strain originates from the Gulf of Alaska.

The mol% G + C of the DNA is: 45.8 (T_m)

Deposited strain: NW430, Nm 55.

Editorial Note: Readers are advised that although the species of *Nitrosomonas* listed here appeared on Validation List No. 83 (IJSEM 2001, *51:* 1945) type material was not deposited in two public service collections as required by Rule 27 and 30 of the Bacteriological Code as amended in 1999. As such, these names may be illegitimate and invalid, and their usage may be called into question until this matter is satisfactorily addressed.

Genus II. **Nitrosolobus** Watson, Graham, Remsen and Valois 1971a, 200[AL]

HANS-PETER KOOPS AND ANDREAS POMMERENING-RÖSER

Ni.tro.so.lob' us. M.L. *nitrosus* nitrous; M.L. n. *lobus* a lobe; M.L. fem. *nitrosolobus* nitrous lobe, a lobe producing nitrite.

Pleomorphic, lobate cells (Fig. BXII.β.93a) **that divide by constriction**. Gram-negative cell wall. Motile cells possess 1–20 **peritrichous flagella**, each 15 nm wide and 2.5–5.0 μm long. **Cells are partially compartmentalized by intracytoplasmic membranes** (Fig. BXII.β.93b). Carboxysomes are not observed. Most, but not all, strains are **urease positive**. **Glycogen inclusions and polyphosphates** are located primarily in the peripheral compartments of the cell. Commonly distributed in agricultural amended soils. Occasionally observed in freshwater environments.

The mol% G + C of the DNA is: 53.2–56.5.

Type species: **Nitrosolobus multiformis** Watson, Graham, Remsen and Valois 1971a, 200.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *NITROSOLOBUS*

Besides the type species, *N. multiformis*, at least one other species exists. It is distinguishable from the type species by a mol% G + C content of DNA of 56.5 and by its significantly smaller cells (0.8–1.2 × 1.0–1.5 μm). In contrast to *N. multiformis*, this species is occasionally found in sewage disposal plants. This second species of the genus *Nitrosolobus* has not yet been named.

List of species of the genus Nitrosolobus

1. **Nitrosolobus multiformis** Watson, Graham, Remsen and Valois 1971a, 200.

mul.ti.for' mis. L. adj. *multus* many; L. n. *forma* shape; M.L. adj. *multiformis* many shapes.

The general characteristics are the same as those described for the genus. Cells are 1.0–1.2 × 1.0–2.5 μm.

The mol% G + C of the DNA is: 53.2–54.6 (Bd, T_m).
Type strain: C-71, ATCC 25196.
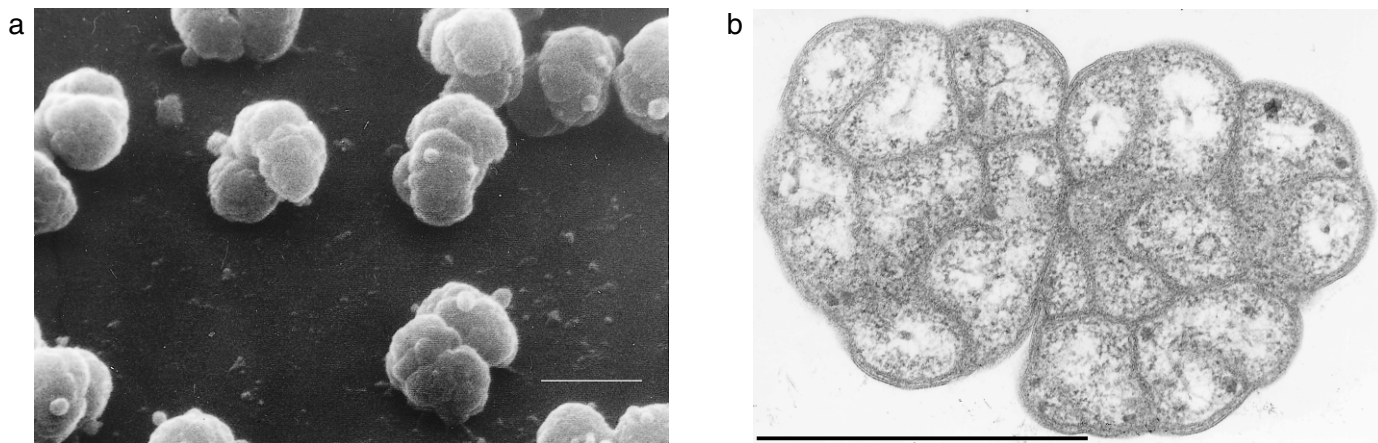*GenBank accession number (16S rRNA)*: L35509.

FIGURE BXII.β.93. (*a*) Scanning electron micrograph and (*b*) electron micrograph of a thin section of cells of *Nitrosolobus multiformis* and *Nitrosolobus* sp. N1 5, respectively. Bars = 1 μm.

Genus III. **Nitrosospira** Winogradsky and Winogradsky 1933, 406[AL]

HANS-PETER KOOPS AND ANDREAS POMMERENING-RÖSER

Ni.tro.so.spi' ra. M.L. adj. *nitrosus* nitrous; Gr. n. *spira* a coil, spiral; M.L. fem. n. *Nitrosospira* nitrous spiral.

Cells are tightly coiled spirals (Fig. BXII.β.94a), 0.3–0.4 μm in width, with 3–20 turns. Occasionally, vibrioid cells occur in cultures. By phase-microscopy, **spherical forms** 0.8–1.2 μm in diameter may be observed in cultures. Gram-negative cell wall. **Intracytoplasmic membranes are rare** (Fig. BXII.β.94b), but tubular invaginations are observed. Motile strains possess **peritrichous flagella**. 1–6 flagella are observed, each 3–5 μm in length. Urease-positive strains, as well as urease-negative strains, exist in all species. Common in grasslands, heath, forest soils, and mountainous environments. Some strains have been isolated from building stones and from acid soils (pH 4.0–4.5). Occasionally observed in freshwater environments. Using molecular ecological methods, the existence of *Nitrosospira*-like groups in marine environment has repeatedly been indicated (Stephen et al., 1996; Phillips et al., 1999; Bano and Hollibaugh, 2000). However this has not yet been proven via isolation of strains.
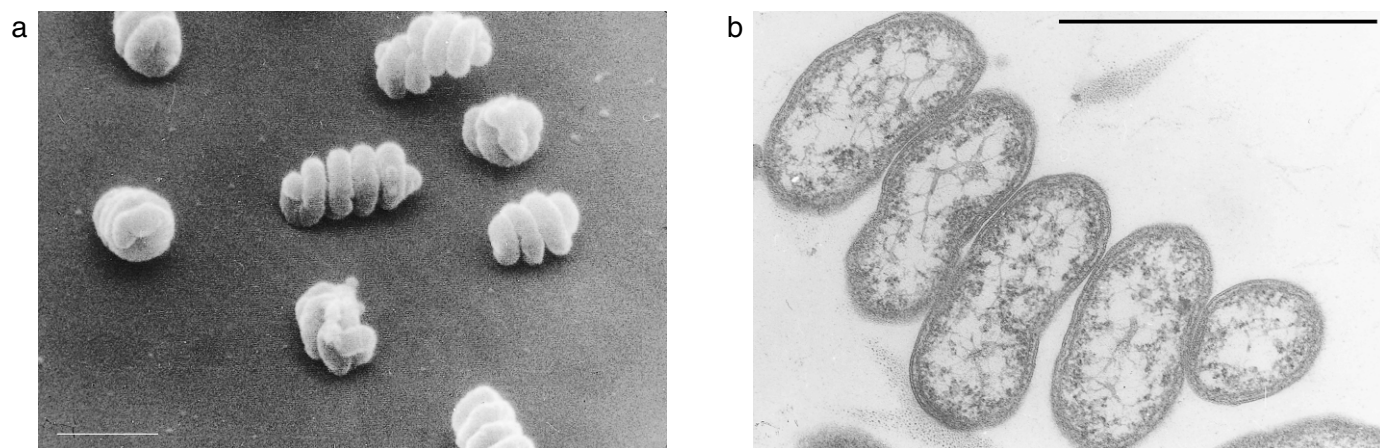
FIGURE BXII.β.94. (*a*) Scanning electron micrograph and (*b*) electron micrograph of a thin section of cells of *Nitrosospira briensis* and *Nitrosospira* sp. Nsp 17, respectively. Bars = 1 μm.

The mol% G + C of the DNA is: 53.2–55.4.

Type species: **Nitrosospira briensis** Winogradsky and Winogradsky 1933, 407.

TAXONOMIC COMMENTS

Originally, two species, *N. briensis* and *"N. antarctica"*, were described by Winogradsky and Winogradsky (1933). Since significant differential characteristics were missing, *"N. antarctica"* was stated by Watson (1971a) to be a subjective synonym of *N. briensis*. However, in DNA reassociation experiments, Koops and Harms (1985) have demonstrated that at least five species of the genus *Nitrosospira* exist. Two groups, containing three and two species, respectively, could be distinguished with 52–53 and 55–56 mol% G + C of their DNA. However, new species have not been described because further phenotypic differential characteristics have not yet been determined.

List of species of the genus Nitrosospira

1. **Nitrosospira briensis** Winogradsky and Winogradsky 1933, 407.

   bri.en′ sis. N.L. adj. *briensis* of Brie, a French place name.

   General characteristics are those described for the genus. The original type strain has not been preserved as laboratory culture.

   The mol% G + C of the DNA is: 53.8–54.1 (T_m, Bd).

   Type strain: C-76.

Genus IV. "**Nitrosovibrio**" *Harms, Koops and Wehrmann 1976, 110*

HANS-PETER KOOPS AND ANDREAS POMMERENING-RÖSER

Ni.tro.so.vib′ ri.o. M.L. adj. *nitrosus* nitrous; L. v. *vibrio* to move rapidly to and from, to vibrate; M.L. masc. n. *Nitrosovibrio* a vibrio producing nitrite.

Slender, curved rods (Fig. BXII.β.95a), 0.3–0.4 × 1.1–3.0 μm. **Spherical forms**, 1.0–1.2 μm in diameter, may occur in cultures. Gram-negative cell wall. **Intracytoplasmic membranes are rare** (Fig. BXII.β.95b), but when present, appear as tubular invaginations. Motile cells possess 1–4 **subpolar to lateral flagella** (Fig. BXII.β.96), about 18 nm wide and 4.2–7.5 μm long. Carboxysomes observed in one strain. Most, but not all, strains are **urease positive**. Commonly distributed in oligotrophic soils, such as grasslands, heath, and forest soils, as well as in mountainous environments. Some isolates originate from acid tea soils and from building stones.

The mol% G + C of the DNA is: 53.9.

Type species: "**Nitrosovibrio tenuis**" Harms, Koops and Wehrmann 1976, 110.

TAXONOMIC COMMENTS

Besides the type species, at least one further species exists, but has not yet been distinguished by phenotypic characters or named.
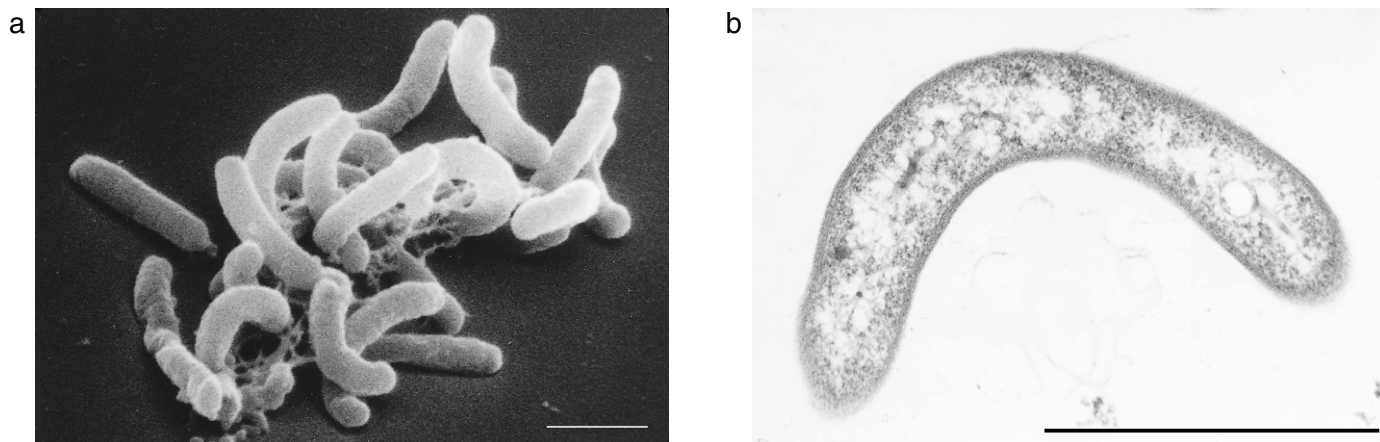
FIGURE BXII.β.95. (*a*) Scanning electron micrograph and (*b*) electron micrograph of a thin section of cells of *"Nitrosovibrio tenuis"* and *"Nitrosovibrio"* sp. Nv 12, respectively. Bars = 1 μm.

List of species of the genus "Nitrosovibrio"

1. **"Nitrosovibrio tenuis"** Harms, Koops and Wehrmann 1976, 110.

te′ nu.is. L. adj. *tenuis* slender.

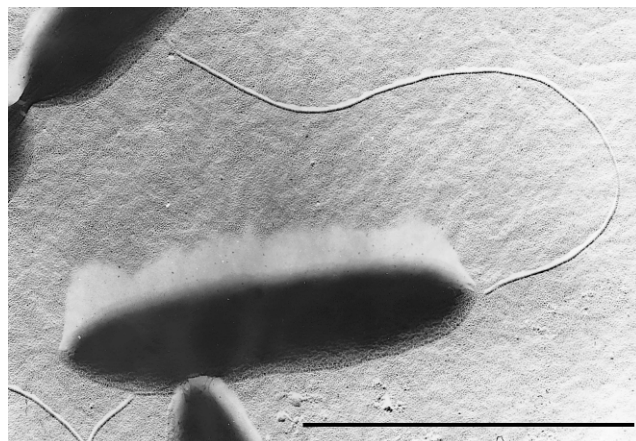Characteristics are the same as those described for the genus.

Deposited strain: Nv 1.

FIGURE BXII.β.96. Electron micrograph of a *"Nitrosovibrio tenuis"* cell shadowed with chromium and showing a single subpolar flagellum. Bar = 1 μm.

Family II. **Spirillaceae** Migula 1894, 237[AL]

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Spi.ril.la′ ce.ae. M.L. dim. neut. n. *Spirillum* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Spirillaceae* the *Spirillum* family.

The family *Spirillaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the family contains the genus *Spirillum* (type genus).

Description is the same as for the genus *Spirillum*.
Type genus: **Spirillum** Ehrenberg 1832, 38.

Genus I. **Spirillum** Ehrenberg 1832, 38[AL]

NOEL R. KRIEG

Spi.ril′ lum. Gr. n. *spira* a spiral; M.L. dim. neut. n. *Spirillum* a small spiral.

Rigid, helical cells, 1.4–1.7 × 14–60 μm. A **polar membrane** underlies the cytoplasmic membrane at the cell poles and is visible in ultrathin sections. **Intracellular poly-β-hydroxybutyrate granules are formed.** Coccoid bodies are not formed. Gram negative. **Motile by large bipolar tufts of flagella** having a long wavelength and about one helical turn; these are composed of approximately 75 flagella and are easily visible by dark-field or phase-contrast microscopy. **Microaerophilic** in ordinary liquid

media, but can grow aerobically in special media or with certain supplements. Colonies on solid media can be obtained only under special conditions. Have a **strictly respiratory type of metabolism** with oxygen as the terminal electron acceptor. Growth does not occur anaerobically with nitrate. Optimal temperature, 30°C. **Oxidase and phosphatase positive. Catalase negative.** Indole and sulfatase negative. Casein, starch, esculin, gelatin, DNA, and RNA are not hydrolyzed. Inhibited by extremely low levels of hydrogen peroxide in the culture medium. NaCl levels above 0.02% are inhibitory. Phosphate levels greater than 0.01 M are inhibitory. **Carbohydrates are not catabolized.** The salts of certain organic acids are used as carbon sources; succinate is used especially well. Vitamins are not required. Occur in **stagnant, freshwater environments**.

The mol% G + C of the DNA is: 38 or 36.

Type species: **Spirillum volutans** Ehrenberg 1832, 38.

FURTHER DESCRIPTIVE INFORMATION

Structural features   An unusual elaboration of the plasma membrane, the "polar membrane," occurs in *S. volutans*. It is attached to the inside of the plasma membrane by barlike links and is located in the region surrounding the polar flagella. Such a membrane has been found mainly in genera of helical bacteria, such as *Aquaspirillum, Campylobacter, Ectothiorhodospira,* and *Rhodospirillum.* In *Campylobacter,* it is an assembly of ATPase molecules at the poles of the cell (Brock and Murray, 1988). Intracellular poly-β-hydroxybutyrate occurs in the form of prominent granules which are refractile by phase-contrast microscopy and which stain with metachromatic stains such as Ponder's stain (Wells and Krieg, 1965) or with the fluorescent dye Nile blue A (Ostle and Holt, 1982).

Motility   The cells are actively motile and swim in straight lines with frequent reversal of direction. The bipolar flagellar fascicles are exceptionally large and consist of many individual flagella (Fig. BXII.β.97). As noted by Metzner (1920), during its rotation the fore fascicle appears to describe a wide bell which is opened toward the rear of the cell; the aft fascicle extends behind the cell and appears to describe a wide goblet (Fig. BXII.β.98). There is no true anterior cell pole: when the fascicles change their orientation the cell reverses its direction of swimming (Fig. BXII.β.98). Because of the wide zones of rotation of the fascicles, Metzner believed that the mechanical effect of the flagella was mainly indirect, i.e., to cause an opposite rotation of the cell body, which, because of its helical shape, would then screw through the medium. Winet and Keller (1976) provided evidence that the aft fascicle of helical cells beats in a helical fashion just as other bacterial flagella do, and Padgett et al. (1983) reported that straight mutant cells could swim at nearly the same speed as helical cells. Thus, it is likely that the flagella operate in a manner similar to that of other bacterial flagella and that the bell and goblet conformations are merely due to the long wavelength of the flagella. Ramia and Swan (1994) have used high-speed cinemicrography to record the swimming of unipolarly flagellated cells. The geometry of these cells was numerically modeled with curved isoparametric boundary elements (from the measured geometrical parameters), and an existing boundary element method (BEM) program was applied to predict the mean swimming linear and angular speeds. Inhibition of the motility of *S. volutans* by low levels of various heavy metals and other toxicants led to development of a method for the monitoring of pollutants in industrial effluents (Bowdre and Krieg, 1974). (See Dutka et al. (1983), Goatcher et al. (1984), Moore (1984), Cortes et al. (1996), Ghosh et al. (1996) and Lacava and Ortolono (1997) for applications, modifications, and evaluations.)

Cultivation   *S. volutans* is a microaerophile but can be cultivated easily in a semisolid medium, such as MPSS broth or CHSS
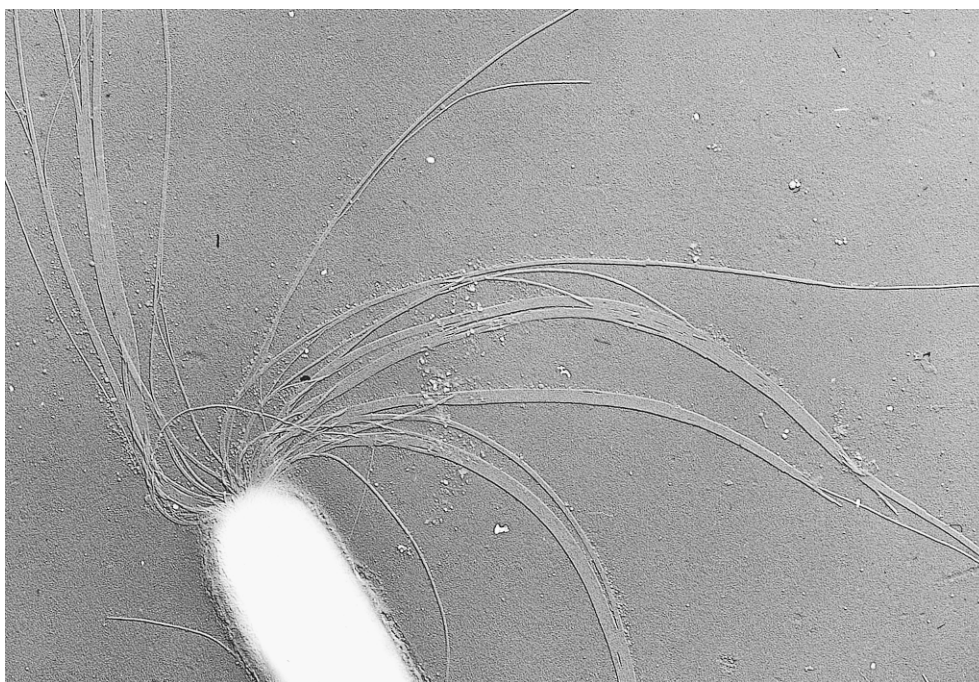

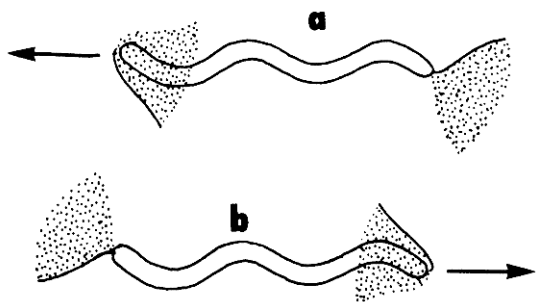**FIGURE BXII.β.97.** Flagellar fascicle of *Spirillum volutans* (× 13,000).

FIGURE BXII.β.98. Diagram of *Spirillum volutans* showing the orientation of the bipolar flagellar fascicles. (*a*) During swimming the fascicles form oriented cones of revolution. (*b*) During reversal of swimming direction, both fascicles reorient simultaneously. *Arrows* in (*a*) and (*b*) indicate the direction of swimming. (Reproduced with permission from J.H. Bowdre and N.R. Krieg, Virginia Polytechnic Institute and State University Water Resources Research Center Bulletin No. 69, 1974, ©Virginia Water Resources Research Center.)

broth[1] prepared with 0.15% agar, incubated under an air atmosphere. Growth is initiated as a thin band or disc several millimeters or centimeters below the surface of the medium, where the respiratory rate of the cells matches the rate of diffusion of oxygen to the cells. As the cell numbers increase, the band becomes denser and migrates closer to the surface. Dense growth just beneath the surface occurs in 48 h.

In MPSS broth, *S. volutans* grows only when incubated under atmospheres of 1–12% oxygen, despite the occurrence in the cells of a superoxide dismutase (SOD) of the iron type (Cover, 1978; Padgett, 1981). Growth does not occur under anaerobic conditions or under an air atmosphere. Addition of catalase (0.8 U/ml) or bovine erythrocyte SOD (4 U/ml) to MPSS broth allows growth to occur under an air atmosphere with static incubation; the two enzymes are most effective when used in combination (0.08 U/ml each) and exert a synergistic effect (Padgett et al., 1982). Potassium metabisulfite (0.005%), acting in conjunction with the FeCl$_3$ component of the MPSS broth, also permits aerobic growth to occur (Padgett et al., 1982).

Aerobic growth can also be obtained by the use of CHSS broth; turbid cultures are obtained in 24 h in 20 × 125-mm loosely screw-capped tubes incubated in a slanted position under an air atmosphere; daily transfer is required. The pH of the medium rises to 8.0 or higher due to oxidation of the succinate component of the medium. A chemically defined medium for *S. volutans* devised by Bowdre et al. (1976) supports growth under a 6% oxygen atmosphere. When supplemented with norepinephrine (10^{-5} to 10^{-6} M), this medium also supports growth under an air atmosphere.

Growth of *S. volutans* on the surface of solid media (MPSS or CHSS broth solidified with 15.0 g/l agar) is difficult to obtain and depends on several factors: (a) protection of the plates from exposure to illumination during preparation and incubation; (b) addition of potassium bisulfite (0.002%), catalase (130 U/ml),

or SOD (30 U/ml) to the medium (the enzymes must be added aseptically to the molten medium just before dispensing into Petri dishes); and (c) incubation of the plates in an atmosphere of high humidity for 24 h prior to inoculation and for 5 d after inoculation (Padgett et al., 1982). Even with these precautions, colonies have developed only under oxygen atmospheres of 12% or less, and only 22–72% of the cells spread onto the surface of the plates develop into colonies. Colony counts approaching direct microscopic counts have been obtained by inoculating culture dilutions into a semisolid Colony Count Medium (CCM)[2] and using this to overlay a thicker layer of sterile medium, and by supplementing the medium with pyruvate, which destroys hydrogen peroxide. Both the pyruvate and the overlay are necessary for optimal results (Alban and Krieg, 1996). Colonies are pinpoint in size.

Respiration Respiration rates of *S. volutans* suspended in 0.05 M phosphate buffer are very low (Cole and Rittenberg, 1971), but are higher~~and, in fact, comparable to those for aerobes~~when a less inhibitory buffer is used, such as BES buffer (*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid) (Caraway and Krieg, 1974). Succinate supports the highest rate of oxygen uptake; fumarate, malate, oxaloacetate, and pyruvate are also readily oxidized. Lactate, butyrate, and β-hydroxybutyrate are oxidized to a lesser extent, and citrate, aconitate, isocitrate, α-ketoglutarate, aspartate, glutamate, casein hydrolysate, and carbohydrates are oxidized only very slightly or not at all. When placed in a nonnutritive medium, *S. volutans* continues to retain motility for up to 24 h, with intracellular poly-β-hydroxybutyrate serving as an endogenous energy source (Caraway and Krieg, 1974). Cytochromes of the *b*, *c*, and *o* types have been detected in *S. volutans*, as well as cytochrome oxidase, NADH oxidase, and various tricarboxylic acid cycle enzymes (Cole and Rittenberg, 1971). Aerotactic responses of *S. volutans* to self-created oxygen gradients have been described by Wells and Krieg (1965) and Caraway and Krieg (1974).

Sensitivity to hydrogen peroxide *S. volutans* lacks catalase and is extraordinarily sensitive to H$_2$O$_2$. Inhibition of growth occurs by addition of as little as 0.29 μM H$_2$O$_2$ to culture media (Padgett et al., 1982), and the organism is killed rapidly by levels greater than 10 μM (Alban and Krieg, 1998). Exposure of MPSS broth to moderate or strong illumination causes the generation of H$_2$O$_2$ at levels sufficient to inhibit growth, and culture media should be protected from illumination. Potassium bisulfite, and especially a combination of SOD and catalase, can help to prevent the inhibitory effects caused by illumination. A hydrogen peroxide-resistant mutant obtained by single step mutagenesis with diethyl sulfate was reported to survive and grow after exposure to 40 μM H$_2$O$_2$ and had high NADH peroxidase activity, whereas the wild type had no detectable activity; however, the mutant was no more oxygen tolerant than the wild type. The mutant con-

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1. MPSS broth (g/l): Bacto peptone (Difco), 5.0; succinic acid (free acid), 1.0; (NH$_4$)$_2$SO$_4$, 1.0; MgSO$_4$·7H$_2$O, 1.0; FeCl$_3$·6H$_2$O, 0.002; and MnSO$_4$·H$_2$O, 0.002. The pH is adjusted to 6.8 with 2 N KOH before autoclaving. For CHSS broth, 2.5 g of vitamin-free, salt-free acid-hydrolyzed casein (ICN Biochemicals Inc., Aurora, Ohio) is substituted for the peptone component, and 0.1 g NaCl is added (*S. volutans* requires a low level of Na$^+$). Different lots of casein hydrolysate may vary in their ability to support good growth. For semisolid media, 1.5 g of agar is added.

2. CCM medium (g/l): vitamin-free, salt-free CH (ICN Biochemicals Inc., Aurora, Ohio), 2.6 g ; succinic acid (free acid), 1.0; (NH$_4$)$_2$SO$_4$, 1.0 ; MgSO$_4$·7H$_2$O, 1.0; KH$_2$PO$_4$, 0.12; NaCl, 0.04; sodium pyruvate, 0.3; FeCl$_3$·6H$_2$O, 0.002; and MnSO$_4$·H$_2$O, 0.002. The pH is adjusted to 7.3 with KOH, 0.05 g potassium metabisulfite is added, and the pH is readjusted to 7.3. Agar (7 g/l) is added and dissolved by boiling, and the medium is sterilized by autoclaving. The pH of the cooled medium after autoclaving should be 6.8. Fifteen-ml volumes of sterile semisolid medium (CCM plus 0.7% agar) are dispensed into Petri dishes and allowed to gel for 30 min. A 0.1-ml volume of dilution of the cell suspension is inoculated into 10 ml of semisolid CCM at 45°C and is poured onto the plates as an overlay. After this has gelled, the plates are incubated at 30°C in an atmosphere of O$_2$/N$_2$ (6:94) and incubated for 3–4 days.

stitutively expressed a 21.5-kDa protein that showed high relatedness to rubrerythrin and nigerythrin based on amino acid sequence comparison and was undetectable and noninducible in the wild type cells (Alban et al., 1998).

Nutrition The nutritional requirements of *S. volutans* are not well understood, since the defined medium of Bowdre et al. (1976) may not necessarily be a minimal medium. If succinate is omitted from this medium, however, no growth occurs, and omission of any of the amino acids present (threonine, methionine, histidine, isoleucine, and cystine) gives a decreased growth response. A very low level of NaCl is required. The phosphate concentration must be no higher than 10^{-4} M for aerobic growth to occur, although growth under microaerobic conditions will occur if the level is increased to 10^{-2} M. Because of the toxic effects of low levels of heavy metals, glassware used for cultivation of *S. volutans* must be cleaned with acid and washed extensively in tap and distilled water; even growth in CHSS broth is dependent upon the use of exceptionally clean glassware.

Polyamines Using a polyamine-free medium, Hamana et al. (1994) reported that *S. volutans* cells contain putrescine, cadaverine, and spermidine, but not diaminopropane, 2-hydroxyputrescine, or homospermidine.

Habitat *S. volutans* has been isolated from stagnant pond water in Virginia and from the cooling water of a sugar beet refinery in England (Rittenberg and Rittenberg, 1962; Wells and Krieg, 1965); however, the organism is widely distributed in stagnant freshwater sources and can be demonstrated in nearly any hay infusion prepared from such sources. In hay infusions, the organism occurs in greatest numbers just beneath the surface scum (composed of aerobic organisms), presumably at a location where the dissolved oxygen level is most suitable or where hydrogen peroxide is being destroyed by other bacteria.

ENRICHMENT AND ISOLATION PROCEDURES

Initial enrichment from hay infusion is accomplished by inoculation of Pringsheim's soil medium (Rittenberg and Rittenberg, 1962). This medium is prepared by placing one wheat or barley grain in a large test tube, covering the grain with 3–4 cm of garden soil, filling the tube nearly to the top with tap water, and sterilizing in an autoclave. Even with this enrichment, *S. volutans* is vastly outnumbered by other bacteria. At present, the only successful method for isolation is a mechanical method based on the ability of *S. volutans* to out-swim other bacteria. This method, first devised by Giesberger (1936) for isolation of other spirilla, has been successfully used for *S. volutans* by Rittenberg and Rittenberg (1962) and Wells and Krieg (1965).

A capillary is prepared by heating sterile 5-mm glass tubing in a flame, pinching the softened portion with square-ended forceps until almost closed, then reheating the flattened portion and drawing it out. The resulting oval capillary should be 15–30 cm long and 0.1–0.3 mm in diameter. After cooling, the capillary is broken with sterile forceps at the tip and 10–20 cm of sterile medium (the supernatant fluid from Pringsheim's soil medium) are drawn into it, followed by 2–4 cm of enrichment culture. There should be no air space between the sterile medium and the culture. The tip is then sealed in a flame, leaving a small air space at the tip of the capillary. The capillary is mounted horizontally on the stage of a microscope and observed at 100X. Because of its rapid motility, *S. volutans* will often be able to outdistance the contaminants and be the first to arrive in the distal portion of the sterile medium. (*S. volutans* will frequently form a band of cells that migrates along the capillary in response to a self-created oxygen gradient; however, the migration rate of such a band is relatively slow, and it is more fruitful to watch for faster-swimming cells well in advance of such a band.) As soon as some spirilla have entered the distal regions of the sterile medium, the capillary is broken behind them and sealed in a flame. After the outside of the capillary has been sterilized with strong hypochlorite solution, and the latter removed with sterile thiosulfate solution, the tip is broken and the spirilla are expelled into a suitable medium. The medium used by Rittenberg and Rittenberg (1962) and Wells and Krieg (1965) was sterile Pringsheim's soil medium contained in a dialysis sac which was suspended in a mixed culture of other bacteria; however, it seems likely that simply expelling the spirilla into a tube of semisolid MPSS or CHSS medium might be a satisfactory alternative. Purity of the cultures is verified by microscopic examination.

MAINTENANCE PROCEDURES

S. volutans may be maintained in semisolid MPSS or CHSS medium at 30°C with transfer every 4–5 days. Cultures may also be maintained in CHSS broth (incubated statically under an air atmosphere) at 30°C with daily transfer.

Preservation by lyophilization has not yet been possible, but cultures may be preserved indefinitely in liquid nitrogen (Pauley and Krieg, 1974). Cells from a broth culture are harvested at $3500 \times g$, washed once with sterile nutrient broth, and suspended to a dense concentration in nutrient broth containing 10% (v/v) DMSO. After incubation for 30 min to allow penetration of the cells by the cryoprotective agent, the suspension is dispensed into vials. After sealing, the vials are frozen in a mixture of dry ice and alcohol and stored by submersion in liquid nitrogen.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

The characteristics listed in Table BXII.β.106 were determined by methods described by Hylemon et al. (1973a). These authors employed PSS broth (containing 1.0% peptone) rather than MPSS broth, but the latter medium is more satisfactory. Cultures in liquid media are incubated under an atmosphere containing 6% oxygen; this oxygen level is most easily obtained by exhausting the air from the culture vessel and refilling the vessel several times with a mixture of O_2/N_2 (6:94). An alternative procedure is to exhaust the air in the culture vessel until the pressure becomes 0.29 atm, then fill the vessel with N_2 to 1 atm. For certain biochemical tests such as phosphatase activity, the surface of plates of PSS (or MPSS) medium containing 0.7% agar is inoculated heavily (to give confluent growth) and the plates are incubated in a humid atmosphere under 6% O_2.

DIFFERENTIATION OF THE GENUS *SPIRILLUM* FROM OTHER GENERA

Table BXII.β.107 lists the features that distinguish the genus *Spirillum* from other genera of chemoheterotrophic, microaerophilic, motile, curved bacteria.

The large size of the cells, the ease with which the flagellar fascicles can be seen by phase microscopy, and the microaerophilic nature of the organism, serve to differentiate the genus from chemotrophic freshwater spirilla in the genus *Aquaspirillum*. Inhibition of growth by NaCl differentiates the genus from members of *Oceanospirillum*.

The genus *Campylobacter* exhibits certain similarities to *Spirillum*, in that both genera contain cells with polar flagella and are microaerophilic, with a strictly respiratory type of metabolism.

Neither genus can catabolize carbohydrates. The DNA base composition is similar (32–35 mol% G + C for *C. fetus*; 38 mol% G + C for *S. volutans*). Moreover, campylobacters, although nominally vibrioid in shape, can often exhibit a spirillum-like appearance, and, like *S. volutans*, *Campylobacter* species have a polar membrane. Despite these similarities, there are marked differences between the two species: *S. volutans* has a much greater cell diameter, large bipolar tufts of flagella rather than a single flagellum at one or both poles, does not form coccoid bodies, and is not associated with animals or humans.

Distinctive morphological similarities exist between *S. volutans* and members of the phototrophic genus *Thiospirillum*. *Thiospirillum jenense* has flagellar fascicles which are remarkably similar to those of *S. volutans*, and both species have cells of large diameter. However, *T. jenense* is obligately phototrophic and anaerobic, and has a higher mol% G + C for its DNA (45%).

"*Aquaspirillum voronezhense*" has been described by Grabovich et al. (1987) and has a cell diameter of 1.5 × 3.0 μm and large flagellar fascicles with up to 50 flagella, as well as a number of other features similar to those of *S. volutans*; however, it is aerobic, catalase- and urease-positive, forms colonies on PSS medium under aerobic conditions, and has a mol% G + C of the DNA of 58–60.

TAXONOMIC COMMENTS

At present, the genus *Spirillum* is represented by only a single species, *Spirillum volutans*, of which only two isolates are available. *S. volutans* is a member of the *Betaproteobacteria* and the family *Spirillaceae*, based on the *Bergey's Manual* revision of the RDP tree.

The two species "*Spirillum minus*" and "*Spirillum pulli*" do not belong to the genus *Spirillum* and are described under *Species Incertae Sedis*.

List of species of the genus Spirillum

1. **Spirillum volutans** Ehrenberg 1832, 38[AL]

    vo'lu.tans. L. v. *voluto* to tumble about; L. part. adj. *volutans* tumbling about.

    The morphological characteristics are as described for the genus, listed in Tables BXII.β.106 and BXII.β.107, and depicted in Figs. BXII.β.98 and BXII.β.99. Isolated from stagnant freshwater sources.

    The mol% G + C of the DNA is: 38 (T_m) or 36 (Bd).
    Type strain: ATCC 19554.
    GenBank accession number (16S rRNA): M34131.
    Additional Remarks: Reference strain ATCC 19553.

Other Organisms

1. "*Sporospirillum*" Delaporte 1964, 257.

    spo.ro.spi.ril'lum. Gr. n. *sporos* a seed (spore); Gr. n. *spira* a spiral; M.L. dim. n. *spirillum* a small spiral; M.L. neut. n. *Sporospirillum* a small spore (-forming) spiral.

    Rigid, helical bacteria of enormous size, 1.8–4.8 × 40–100 μm. Structures that morphologically resemble endospores occur within the cells, but their thermal resistance has not been determined. The sporelike structures have the ability to rotate and to migrate within the cytoplasm of the bacteria. They initially develop near the cell poles and later migrate to the center where they are released after the cell ruptures and disintegrates. The Gram reaction has not been reported. The cells are motile, but no organs of locomotion are evident. The relationship of the cells to oxygen is unknown. Occur in the intestinal contents of tadpoles. Have not been isolated. No type species designated.

    a. "*Sporospirillum praeclarum*" (Collin 1913) Delaporte 1964, 259 (*Spirillum praeclarum* Collin 1913, 62.)

    prae.cla'rum. L. adj. *praeclarum* distinguished, famous.

    Cells 3.0–4.0 × 50–100 μm. Diameter of helix, 5–10 μm. Wavelength, 17–23 μm. A single endospore is present, 3–4 × 9–12 μm.

    b. "*Sporospirillum gyrini*" Delaporte 1964, 259.

    gy.ri'ni. L. n. *gyrinus* a tadpole; L. gen. n. *gyrini* of a tadpole.

    Cells 1.8–2.6 × 40–100 μm. Diameter of helix 3–6 μm. Wavelength, 13–20 μm. A single endospore is present, 2 × 5–7 μm.

    c. "*Sporospirillum bisporum*" Delaporte 1964, 260.

    bi.spo'rum. L. adv. *bis* twice; G. n. *sporos* a seed; M.L. gen. pl. n. *bisporum* of two seeds (spores).

    Cells 3.5–4.8 × 50–90 μm. Diameter of helix, 11–15 μm. Wavelength, 27–35 μm. At each pole an endospore occurs, 2–4 × 10–14 μm.

Species Incertae Sedis

1. "**Aquaspirillum voronezhense**" Grabovich, Churikova, Chernykh, Kononyhina and Popravko 1987, 666.

    vo.ro.ne.zhen'se. M.L. adj. *voronezhense* pertaining to the town of Voronezh.

    This species has cells that resemble *Spirillum volutans* in size but the mol% G + C of the DNA differs markedly. Consequently, the placement of this species is uncertain.

    Helical cells 1.5–3.0 μm wide, with 1–3 turns per cell. Diameter of helix, 2.9–6.8 μm. Motile by means of bipolar flagellar fascicles, each fascicle being composed of up to 50 flagella. Gram negative. Poly-β-hydroxybutyrate and volutin are accumulated within the cells. S^0 is accumulated in the presence of sulfides. Colonies are flat and nonpigmented with a diameter of 1–4 mm. Aerobic. Optimal pH for growth, 7.2–8.5; pH range for growth, 6.0–9.0. No growth occurs in the presence of 3% NaCl. Optimal temperature for growth, 28°C. Chemoorganotrophic. Carbohydrates are not utilized. A wide range of organic acids, including acetate, succinate, malate, fumarate, benzoate, α-ketoglutarate, oxaloacetate, pyruvate, salicylate, lactate, and glyoxylate, can be utilized as carbon sources. Citrate, ethanol, glycerol, butanol, and mannitol are not utilized. Utilization of isocitrate, aconitate, oxalate, and formate differs among strains. Aspartate can be utilized as a carbon source; utilization of proline, tyrosine, histidine, glycine, glutamine, and valine differs among strains. Tryptophan, methionine, serine, lysine, phenylalanine, asparagine, cysteine, cystine, alanine, leucine, valine, arginine, and ornithine are not utilized. Vitamins are required. Sources of nitrogen used by the type strain include ammonium salts, casein hydrolysate, yeast extract, peptone, aspartate, glutamate, cysteine; utilization of serine differs among strains. Nitrate, nitrite, and methionine are not utilized. Starch, casein, and gelatin

TABLE BXII.β.106. Characteristics of *Spirillum volutans*

Characteristics	Reaction or Result
Cell diameter, μm[a]	1.4–1.7
Wavelength, μm[a]	16–28
Diameter of helix, μm[a]	5–8
Length of helix, μm[a]	14–60
Number of turns[a]	1–5
Intracellular poly-β-hydroxybutyrate granules present[a]	+
Coccoid bodies present in older cultures[a]	−
Bipolar tufts of flagella present[a]	+
Oxidase (moistened test disc inoculated with centrifuged cells)[a]	+
Catalase[b]	− [c]
Phosphatase (0.01% phenolphthalein diphosphate)[d]	+
Sulfatase (0.01% phenolphthalein disulfate)[d]	−
H₂S from 0.2% cysteine (detector strip)[a]	+
Liquefaction of 12% gelatin[a]	−
Hydrolysis of casein (single-strength milk)[d]	−
Hydrolysis of esculin[a]	−
Hydrolysis of 0.1% DNA or RNA (clear zone after acidification)[d]	−
Indole production from 0.1% tryptophan[a]	−
Hydrolysis of 10% soluble starch[d]	−
Aerobic reduction of 0.1% KNO₃[a]	−
Aerobic reduction of 0.1% H₂SeO₂ (by pink color)[a]	−
Visible growth with 1% bile or 1% glycine[a]	−
Anaerobic growth with 0.1% KNO₃ (sealed with petrolatum)[b]	−
Acid reaction from carbohydrates (38 compounds tested)[e]	−
Urease[f]	−
Mol% G + C of DNA (T_m)	38

[a]Basal medium is PSS broth.

[b]Basal medium is PSS broth + 0.15% agar.

[c]When a few drops of 3% H_2O_2 are added, a few bubbles of oxygen form after 30-min incubation. However, this reaction is probably attributable to the alkalinity of the cultures rather than to catalase activity.

[d]Basal medium is PSS broth + 0.7% agar.

[e]Basal medium is PSS broth lacking succinate and with peptone decreased to 0.2%; 0.0018% phenol red indicator added.

[f]Cells suspended in distilled water to a dense, milky concentration; 0.5 ml added to 2.0 ml of the following medium: BES buffer, 0.1065%; urea, 2.0%; phenol red, 0.001%; pH 7.0. Controls without urea are used. The test is incubated for 24 h.

are not hydrolyzed. Nitrates are not reduced to nitrites. Anaerobic growth does not occur with nitrate, sulfate, thiosulfate, or fumarate as terminal electron acceptors. Catalase, oxidase, and urease positive. Indole negative. H₂S is formed from cysteine. Colored products are formed in media containing benzoate. Source of isolates: sludge in a purification system air tank for the treatment of domestic sewage.

The mol% G + C of the DNA is: 58.5–60.0 (method unknown).

Deposited strain: D-419.

Additional Remarks: Reference strain D-420.

2. **"Spirillum minus"** Carter 1888, 47 (*Spirillum minor* (sic) Carter 1888, 47.)

mi′ nus. L. neut. adj. *minus* less, smaller.

"Spirillum minus" and *"Spirillum pulli"* (below) do not belong to the genera *Aquaspirillum*, *Oceanospirillum*, or *Spirillum*, and their placement is uncertain. Studies of these species have been hampered by lack of reproducible *in vitro* cultivation methods. They do not appear on the *Approved*

Lists of Bacterial Names because no type or reference strains are available, and the organisms are not well characterized. The disease syndromes caused by these species are distinct and recognizable, however. If possible, neotype strains should be designated and either maintained by animal passage or preserved in a recognized culture collection.

Rigid cells; usually described as spiral with two or three turns, although the waves have been reported to be planar (McDermott, 1928). The ends of the cell may be blunt or pointed. Cell size ~0.2 × 3–5 μm; wavelength, 0.8–1.0 μm. Actively motile by one or more flagella at each pole.

Causes one of the two forms of rat-bite fever in man. The disease caused by *"S. minus"* is often termed "Sodoku"; it occurs worldwide but has its greatest frequency in the Far East. The organisms are usually transmitted to humans through the bite of an infected rat, although mice, squirrels and rodent-ingesting animals such as cats, dogs, ferrets, and weasels have also been implicated. *"S. minus"* appears to be a natural parasite of rats, which act as carriers; the infection is usually not lethal in rats. The natural infection frequency for rats varies from country to country but may be as high as 25% (see Babudieri, 1973, for pertinent literature).

The clinical aspects of rat-bite fever and the distinctions between the form caused by *"S. minus"* and that caused by *Streptobacillus moniliformis* have been summarized by Joklik et al. (1980) and by Rogosa (1980). Experimental infections of humans and animals by *"S. minus"* have been described by Babudieri (1973).

"S. minus" is best observed in blood or exudates from patients by dark-field or phase-contrast microscopy of wet mounts; staining with Giemsa or Wright's stain or by silver impregnation is also useful.

"S. minus" is cultured *in vivo* by intraperitoneal inoculation of patients' blood or exudates from lesions, or blood from naturally infected rats, into spirillum-free mice or guinea pigs (Rogosa, 1980); mice are the animals most susceptible to *"S. minus"* infection (Babudieri, 1973). It is questionable whether the organism has ever been cultured successfully in artificial media. Numerous attempts have failed, and various claims of successful cultivation have been unable to be confirmed. One report that may indicate successful cultivation is that of Hitzig and Liebesman (1944), who inoculated blood from a patient into 2% dextrose–veal infusion broth and into 10% tomato extract–veal infusion broth. The addition of citrated human or rabbit blood was required for successful subculturing; also, the organisms initially required incubation in a candle jar but eventually were able to grow aerobically after five months of serial transfer. Confirmation of this report is needed. Considering the morphology, pathogenicity, and sources of *"S. minus"*, serious attention should be given to the possibility that the organism might belong to, or be related to, the genus *Campylobacter*, and the microaerophilic techniques employed for campylobacters might also prove useful for *"S. minus"*.

Deposited strain: none.

3. **"Spirillum pulli"** Mathey 1956, 745.

pul′ li. L. gen. n. *pulli* of a young chicken.

Rigid spiral cells. By dark-field microscopy, the cell size is ~1 × 5–12 μm. Actively motile by means of a single flagellum at each end of the cell.

Believed to be the cause of a diphtheroid stomatitis in the mouths of adult chickens. The lesions are yellowish

TABLE BXII.β.107. Differential features of the genus *Spirillum* and other chemoheterotrophic, motile, aerobic or microaerophilic, Gram-negative vibrioid or helical bacteria[a]

Characteristic	Aquaspirillum	"Aquaspirillum voronezhense"	Arcobacter	Azospirillum	Bdellovibrio	Campylobacter	Cellvibrio	Halomonas variabilis	Helicobacter	Herbaspirillum	Marinomonas communis
Predominant shape:											
Helical	+[b]	+	−	−	−	−	−	−	−	−	−
Curved in one plane	−	−	+	−	+	−	+	+	−	−	+
Vibrioid	−	−	−	+[c]	−	+[d]	−	−	+[e]	+[f]	−
Cell diameter, μm	0.2–1.4	1.5–3.0	0.2–0.9	0.9–1.5	0.2–0.5	0.2–0.9	0.2–0.5	0.5–0.8	0.5–1.0	0.6–0.7	0.7–1.5
Cultivable on inanimate laboratory media	+	+	+	+	+	+	+	+	+	+	+
Require host or host cells for cultivation	−	−	−	−	−[g]	−	−	−	−	−	−
Predacious on other Gram-negative bacteria	−	−	−	−	+[g]	−	−	−	−	−	−
Grow in periplasmic space of host cell	−	−	−	−	+	−	−	−	−	−	−
Exoparasitic growth	−	−	−	−	−	−	−	−	−	−	−
Predacious on eucaryotic algae											
Structures morphologically resembling endospores are present											
Usual arrangement of polar flagella:											
Monotrichous	−	−	+	+	+	−	−	+	−	−	+
Bipolar tufts	+[h]	+	−	−	−	−	−	−	−	−	−
1 at one or both poles	−	−	−	−	−	+[i]	−	−	−	−	−
1–3 at one or both poles	−	−	−	−	−	−	−	−	−	+	−
Multiple at one or both poles	−	−	−	−	−	−	−	−	+[j]	−	−
1 at each pole											
1 or more at each pole	−	−	−	−	−	−	+[l]	−	−	−	−
Sheathed flagella present	−	−	−	−	+	−	−	−	D[k]	−	−
Lateral flagella occur in addition to polar flagella	−	−	−	D[l]	−	−	−	−	D[m]	+	−
Optimal growth temperature 5–9°C; maximum temperature 20°C	−[n]										
Pathogenic for humans or animals	−	−	D	−	−	D	−	−	+	−	−
Inhibited by 3.5% NaCl	+	+	−	D	D	D	−	−	+	+	−
Sea water or Na⁺ required for growth	−	−	D[o]	−	D[o]	−	+	+	−	−	+
Require at least 7% NaCl for growth; can grow with 28% NaCl	−	−	−	−	−	−	−	+	−	−	−
Exhibit magnetotaxis; contains magnetosomes	D[p]	−	−	−	−	−	−	−	−	−	−
Nitrogenase activity under microaerobic conditions	−	−	−	+	−	−	−	−	−	+	−
Relation to oxygen under non-N₂-fixing conditions:											
Aerobic	+	+	−	+	+	−	+	+	−	+	+
Microaerophilic	−	−	+[q]	−	−	+	−	−	+	−	−
Grows anaerobically by using H₂ or formate as electron donor and fumarate as electron acceptor	−	−	D	−	−	D	−	−	−	−	−
Some carbohydrates catabolized	−[r]	−	−	+	−	−	+		−	+	+

(*continued*)

TABLE BXII.β.107. *(cont.)*

Characteristic	Aquaspirillum	"Aquaspirillum voronezhense"	Arcobacter	Azospirillum	Bdellovibrio	Campylobacter	Cellvibrio	Halomonas variabilis	Helicobacter	Herbaspirillum	Marinomonas communis
Glucose catabolized	−[s]	−	−	D			+	−	−	+	+
Cellulose hydrolyzed		+	−	−			+	−	−		
Urease	+	+	−	−		−[t]	−	+	+[u]	−	−
Habitat:											
Freshwater	+	+	−	−	D	−	−	−	−	−	−
Marine	−	−	−[v]	−	D	−	−	−	−	−	+
Soil	−[w]	−	−	+	D	−	+	−	−	+	−
Within plant roots	−	−	D	+	−	−	−	−	−	+	−
Humans and/or warm-blooded animals	−	−	D	−	−	+	−	−	+	−	−
Intestinal contents of tadpoles	−	−	−	−	−	−	−	−	−	−	−

[a] Symbols: +, all species positive except where noted; −, all species negative except where noted; D, differs among species.

[b] *A. delicatum* is mainly vibrioid.

[c] Some cells in *Azospirillum* cultures are straight rods.

[d] Chains of *Campylobacter* cells may have a helical appearance. *Campylobacter rectus*, *Campylobacter showae*, and *Campylobacter gracilis* are straight rods.

[e] *H. trogontum* and *H. bilis* are fusiform straight rods. *H. bizzozeronii* and *H. felis* cells are long helices. *H. cholecystus* cells are coccoid to short curved rods.

[f] Some cells in cultures of *Herbaspirillum* are helical.

[g] All wild-type strains of *Bdellovibrio* upon initial isolation are dependent on intraperiplasmic growth in susceptible bacterial prey. Mutants capable of axenic growth (prey-independent strains) have been derived from the predacious strains, and some strains are facultative, i.e., capable of growth in the presence or absence of prey cells.

[h] *A. delicatum* has mainly a single flagellum at one pole; *A. polymorphum* has mainly a single flagellum at each pole; and *A. arcticum* has a single polar flagellum.

[i] *Campylobacter gracilis* is nonmotile.

[j] *H. cinaedi*, *H. fennelliae*, *H. hepaticus*, *H. pametensis*, *H. pullorum*, and *H. rodentium* have a single flagellum at one of both poles.

[k] *H. pullorum* and *H. rodentium* do not have sheathed flagella.

[l] Especially (or in the case of azospirilla, only) when cells are grown on solid media.

[m] *H. mustelae* is reported to have multiple lateral flagella in addition to polar flagella.

[n] *A. arcticum* is positive.

[o] *A. nitrofigilis* requires Na^+. Marine bdellovibrios require Na^+.

[p] *A. peregrinum* and some strains of *A. itersonii* have nitrogenase activity. *C. nitrofigilis* has nitrogenase activity.

[q] *Arcobacter* species can grow aerobically but may be microaerophilic on primary isolation. *A. nitrofigilis* can grow aerobically on complex media such as *Brucella* agar.

[r] *A. gracile*, *A. itersonii*, *A. peregrinum*, and *A. arcticum* can catabolize a very restricted variety of sugars.

[s] *A. gracile* can produce acid from glucose aerobically but not anaerobically. *A. itersonii* can produce acid from glucose anaerobically. *A. arcticum* can grow on glucose, fructose, and ribose but not on other carbohydrates.

[t] Some strains of *C. lari* are urease positive.

[u] *H. cinaedi* and *H. fennelliae* are urease negative.

[v] *C. nitrofigilis* occurs in the roots of salt-marsh grasses.

[w] *A. arcticum* occurs in arctic sediments.

[x] "*Sporospirillum*" spp. are motile but no organelles of locomotion have been observed.

[y] *O. pusillum* has mainly a single flagellum at each pole.

[z] Although *Wolinella succinogenes* has been regarded as anaerobic, it is in fact a microaerophile. It is capable of respiring with oxygen when it is provided at low concentrations and cannot grow under an air atmosphere. It can also grow anaerobically by using fumarate, polysulfide, or S^0 as a terminal electron acceptor for anaerobic respiration. For further information see Wolin et al. (1961) and Ringel et al. (1996).

[aa] Occurs in sewage waters.

(continued)

TABLE BXII.β.107. *(cont.)*

Characteristic	*Magnetospirillum*	*Micavibrio*	*Oceanospirillum* (helical species)	*Pseudoalteromonas undina*	*Spirillum*	*Spirillum minus*	*"Spirillum pleomorphum"*	*"Spirillum pulli"*	*Sporospirillum*	*Vampirovibrio*	*Wolinella succinogenes*
Predominant shape:											
Helical	+	−	+	−	+	+	−	+	+	−	−
Curved in one plane	−	−	−	+	−	−	+	−	−	−	−
Vibrioid	−	+	−	−	−	−	−	−	−	+	+
Cell diameter, μm	0.2–0.7	0.25–0.35	0.3–1.4	0.7–1.5	1.4–1.7	0.2	0.7–1.0	1.0	1.4–4.8	0.3	0.5–1.0
Cultivable on inanimate laboratory media	+	−	+	+	+	−	+	+	+	+	+
Require host or host cells for cultivation	−	+	−	−	−	+	−	−	−	+	−
Predacious on other Gram-negative bacteria	−	+	−	−	−	−	−	−	−	−	−
Grow in periplasmic space of host cell	−	−	−	−	−	−	−	−	−	−	−
Exoparasitic growth	−	+	−	−	−	−	−	−	−	−	−
Predacious on eucaryotic algae	−	−	−	−	−	−	−	−	−	+	−
Structures morphologically resembling endospores are present	−	−	−	−	−	−	−	−	+	−	−
Usual arrangement of polar flagella:											
Monotrichous	−	+	−	+	−	−	+	−	−[x]	+	+
Bipolar tufts	−	−	+[y]	−	+	−	−	−	−	−	−
1 at one or both poles	−	−	−	−	−	−	−	−	−	−	−
1–3 at one or both poles	−	−	−	−	−	−	−	+	−	−	−
Multiple at one or both poles	−	−	−	−	−	−	−	−	−	−	−
1 at each pole	+	−	−	−	−	−	−	−	−	−	−
1 or more at each pole	−	−	−	−	−	+	−	−	−	−	−
Sheathed flagella present	−	−	−	−	−	−	−	−	−	−	−
Lateral flagella occur in addition to polar flagella	−	−	−	−	−	−	−	−	−	−	−
Optimal growth temperature 5–9°C; maximum temperature 20°C	−	−	−	−	−	−	+	−	−	−	−
Pathogenic for humans or animals	−	−	−	−	−	+	−	+	−	−	−
Inhibited by 3.5% NaCl	+	−	−	−	+	−	−	−	−	−	−
Sea water or Na⁺ required for growth	−	−	+	+	−	−	−	−	−	−	−
Require at least 7% NaCl for growth; can grow with 28% NaCl	−	−	−	−	−	−	−	−	−	−	−
Exhibit magnetotaxis; contains magnetosomes	+	−	−	−	−	−	−	−	−	−	−
Nitrogenase activity under microaerobic conditions	D	−	−	−	−	−	−	−	−	−	−
Relation to oxygen under non-N₂-fixing conditions:											
Aerobic	−	+	+	+	+	−	+	−	−	+	−
Microaerophilic	+	−	−	−	−	+	−	+	+	−	+[z]
Grows anaerobically by using H$_2$ or formate as electron donor and fumarate as electron acceptor	−	−	−	−	−	−	−	−	−	−	+

(continued)

TABLE BXII.β.107. *(cont.)*

Characteristic	*Magnetospirillum*	*Micavibrio*	*Oceanospirillum* (helical species)	*Pseudoalteromonas undina*	*Spirillum*	*Spirillum minus*	*"Spirillum pleomorphum"*	*"Spirillum pulli"*	*Sporospirillum*	*Vampirovibrio*	*Wolinella succinogenes*
Some carbohydrates catabolized	−		−	+	−	−					−
Glucose catabolized				+							
Cellulose hydrolyzed										−	−
Urease											−
Habitat:											
Freshwater	+	+[aa]	−	−	+	−	−	−		+	−
Marine	−		+	+	−	−	−	−			−
Soil	−		−	−	−	−	+	−	−		−
Within plant roots	−		−	−	−	+	−	+	−	−	−
Humans and/or warm-blooded animals	−		−	−	−	−	−	−	−	−	+
Intestinal contents of tadpoles	−								+	−	−

Symbols: +, all species positive except where noted; −, all species negative except where noted; D, differs among species.

a*A. delicatum* is mainly vibrioid.

bSome cells in *Azospirillum* cultures are straight rods.

cChains of *Campylobacter* cells may have a helical appearance. *Campylobacter rectus*, *Campylobacter showae*, and *Campylobacter gracilis* are straight rods.

d*H. trogontum* and *H. bilis* are fusiform straight rods. *H. bizzozeronii* and *H. felis* cells are long helices. *H. cholecystus* cells are coccoid to short curved rods.

eSome cells in cultures of *Herbaspirillum* are helical.

fAll wild-type strains of *Bdellovibrio* upon initial isolation are dependent on intraperiplasmic growth in susceptible bacterial prey. Mutants capable of axenic growth (prey-independent strains) have been derived from the predacious strains, and some strains are facultative, i.e., capable of growth in the presence or absence of prey cells.

g*A. delicatum* has mainly a single flagellum at one pole; *A. polymorphum* has mainly a single flagellum at each pole; and *A. arcticum* has a single polar flagellum.

h*Campylobacter gracilis* is nonmotile.

i*H. cinaedi*, *H. fennelliae*, *H. hepaticus*, *H. pametensis*, *H. pullorum*, and *H. rodentium* have a single flagellum at one of both poles.

j*H. pullorum* and *H. rodentium* do not have sheathed flagella.

kEspecially (or in the case of azospirilla, only) when cells are grown on solid media.

l*H. mustelae* is reported to have multiple lateral flagella in addition to polar flagella.

m*A. arcticum* is positive.

n*A. nitrofigilis* requires Na^+. Marine bdellovibrios require Na^+.

o*P. peregrinum* and some strains of *A. itersonii* have nitrogenase activity. *C. nitrofigilis* has nitrogenase activity.

p*Arcobacter* species can grow aerobically but may be microaerophilic on primary isolation. *A. nitrofigilis* can grow aerobically on complex media such as *Brucella* agar.

q*A. gracile*, *A. itersonii*, *A. peregrinum*, and *A. arcticum* can catabolize a very restricted variety of sugars.

r*A. gracile* can produce acid from glucose aerobically but not anaerobically. *A. itersonii* can produce acid from glucose anaerobically but not aerobically. *A. arcticum* can grow on glucose, fructose, and ribose but not on other carbohydrates.

sSome strains of *C. lari* are urease positive.

t*H. cinaedi* and *H. fennelliae* are urease negative.

u*C. nitrofigilis* occurs in the roots of salt-marsh grasses.

v*A. arcticum* occurs in arctic sediments.

w*"Sporospirillum"* spp. are motile but no organelles of locomotion have been observed.

x*O. pusillum* has mainly a single flagellum at each pole.

zAlthough *Wolinella succinogenes* has been regarded as anaerobic, it is in fact a microaerophile. It is capable of respiring with oxygen when it is provided at low concentrations and cannot grow under an air atmosphere. It can also grow anaerobically by using fumarate, polysulfide, or S^0 as a terminal electron acceptor for anaerobic respiration. For further information see Wolin et al. (1961) and Ringel et al. (1996).

aaOccurs in sewage waters.

white, rather firm, and adherent to the underlying tissue; often they are symmetrical ovoids, one at each side of the lower jaw. Lesions also occur on the palate, the lower surface of the tongue, on the floor of the mouth, between the larynx and the transverse row of papillae on the tongue, around the larynx, and on the walls of the pharynx. The lesions vary in size from approximately 2 to 20 mm.

Attempts to culture "*S. pulli*" in artificial media have been unsuccessful. Experimental passage of the disease in chickens has been accomplished by contact and by experimental inoculation.

Deposited strain: none.

Family III. **Gallionellaceae** Henrici and Johnson 1935b, 4[AL]

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Gal.li.o.nel.la' ce.ae. M.L. fem. n. *Gallionella* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Gallionellaceae* the *Gallionella* family.

The family *Gallionellaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the family contains the genus *Gallionella* (type genus).

Description is the same as for the genus *Gallionella*.

Type genus: **Gallionella** Ehrenberg 1838, 166.

Genus I. **Gallionella** *Ehrenberg 1838, 166*[AL]

LOTTA E-L. HALLBECK AND KARSTEN PEDERSEN

Gal.li.o.nel' la. M.L. dim. ending *-ella* ; M.L. fem. n. *Gallionella* named for B. Gaillon, a customs agent and zoologist (1782–1839) in Dieppe, France.

Gram-negative, **bean-shaped cells**, usually 0.5–0.8 × 1.6–2.5 μm, that secrete an extracellular **twisted stalk** from the concave side, 0.3–0.5 μm in width and up to 400 μm or more in length. The stalk is composed of numerous 2 nm-wide fibers and is produced under microaerophilic conditions when cells are in late exponential or stationary growth phase. Motile by means of a polar flagellum. **Microaerophilic; chemolithotrophic growth** can be obtained *in vitro* using oxygen and ferrous iron concentration gradients in a salt medium with **CO_2 as sole carbon source** (Table BXII.β.108). **Mixotrophic metabolism** has been demonstrated with glucose, fructose, and sucrose. Can be found where anaerobic **groundwater with ferrous iron** reaches an oxygen-containing environment. Belongs to the **Betaproteobacteria**, family *Gallionellaceae*, with one known species, *G. ferruginea*. Most closely related species according to 16S rDNA sequence analysis is the chemolithotroph *Nitrosospira multiformis*, distantly related with a 16S rDNA sequence similarity of 90%.

The mol% G + C of the DNA is: 51–54.6 (Hanert, 1989).

Type species: **Gallionella ferruginea** Ehrenberg 1838, 166.

FURTHER DESCRIPTIVE INFORMATION

The 16S rRNA gene of *Gallionella ferruginea* (strain Johan) has been sequenced between base numbers 47 and 1405 (*E. coli* numbering) (Hallbeck et al., 1993). Phylogenetic analysis of these sequence data placed *Gallionella* among the *Betaproteobacteria*. *G. ferruginea* is distant from other species in the tree, with a 10% sequence difference compared to the closest species, the chemolithotroph *Nitrosospira multiformis*. The remote position of *G. ferruginea* in relation to other species and its utilization of iron as energy source and electron donor compared to ammonia for the closest gene cluster, *Nitrosomonadaceae*, indicate that a separate family, *Gallionellaceae*, is justified. There is only one named species at this time. The capacity for chemolithotrophic iron oxidation among bacteria has been suggested to be evolutionarily widespread (Lane et al., 1992). This is supported by, for instance, the phylogenetic distance (85% 16S rRNA gene sequence simi-

larity) between *Gallionella* and the iron-oxidizing genus *Thiobacillus*.

The size, shape, and ultrastructure of *Gallionella* are illustrated in Figs. BXII.β.99, BXII.β.100, BXII.β.101, and BXII.β.102. Cells are curved, bean-shaped, and may have a polar flagellum (Fig. BXII.β.99). A twisted extracellular stalk is secreted from the concave side of the cell (Figs. BXII.β.100 and BXII.β.101A). It consists of numerous 2 nm-wide fibers at the point of excretion (Vatter and Wolfe, 1956). The stalk becomes continuously encrusted with precipitated ferric iron oxide (Fig. BXII.β.101B), which may totally cover old stalks. The composition of the stalk has not been conclusively demonstrated, but inorganic as well as organic compositions have been suggested. Hanert (1989) proposed the stalk to consist of colloidal ferric hydroxide, based on its disappearance in 0.12% sodium thioglycolate. Hallbeck and Pedersen (1995) found a higher carbon-to-nitrogen ratio (C/N 6.8) in stalk-forming cultures compared to a non-stalk-forming culture (C/N = 4.3), and concluded the stalk to be composed of an extracellular carbon skeleton without a dominating protein component. The cell wall is Gram-negative (Fig. BXII.β.102D–E).

Two strains of *Gallionella* have been described (Table BXII.β.109). Strain BD from a drainpipe in Braunschweig was described by Hanert (1989) and strain Johan from a 60 m-deep drinking water well was described by Hallbeck and Pedersen (1990, 1991, 1995) and Hallbeck et al. (1993). Strain BD was reported to have intracytoplasmic membranes (Hanert, 1989) while strain Johan does not (Fig. BXII.β.102). The isolation procedure for *Gallionella* is by serial dilution and therefore the possibility of contaminants in the cultures cannot be conclusively excluded. Serial thin sectioning should show a stalk connected to the sectioned cell, as in Figure BXII.β.102A–C, to confirm it to be a cell of *Gallionella*. Inclusions that might be poly-β-hydroxybutyrate have been noted but apart from this, strain Johan does not show any specific intracellular fine structures (Fig. BXII.β.102D–E).
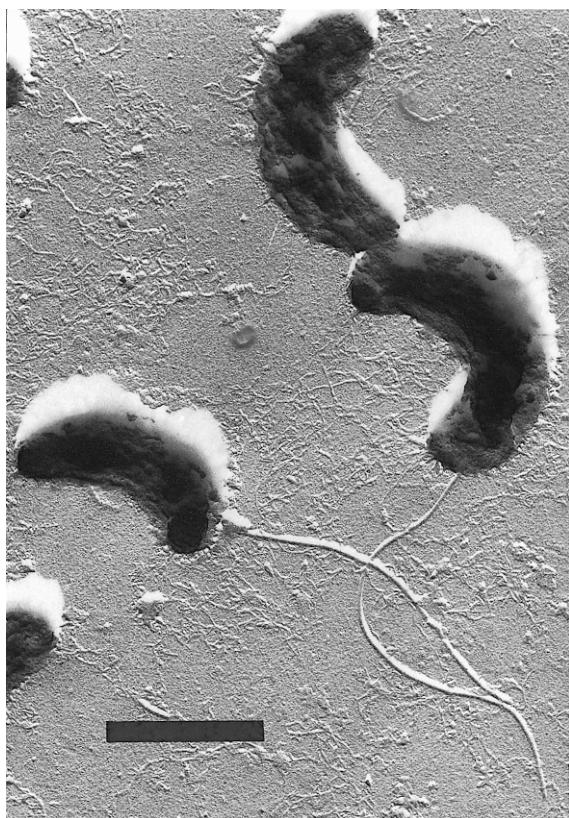
FIGURE BXII.β.99. *G. ferruginea* strain BD cell morphology and flagella arrangement in cell suspension after the stalks were dissolved in sodium thioglycolate. Bar = 1 μm.
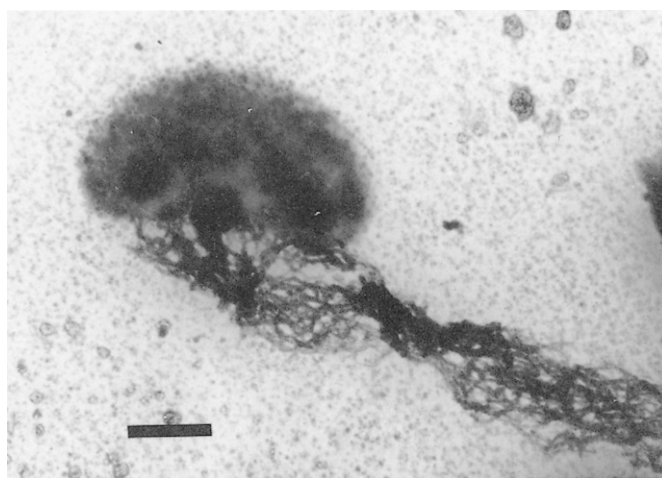

FIGURE BXII.β.100. Apical cell, region of stalk secretion, and ultrastructure of the stalk of attached *G. ferruginea* strain BD from the drain pipe from which it was isolated. Bar = 0.5 μm. (Reproduced with permission from H.H. Hanert, *Archives of Microbiology 60:* 348–376, 1968, ©Springer-Verlag, Berlin.)

Gallionella can be cultured *in vitro* in screw-capped test tubes using oxygen and ferrous iron concentration gradients in a salt medium with carbon dioxide as sole carbon source (Fig. BXII.β.103). Solid-phase ferrous iron is placed on the bottom of the tube in a fresh, autoclaved, and oxygen-free salt medium. With this procedure, the concentration of ferrous iron will decrease from the bottom to the top of the tube as it dissolves and diffuses away from the solid phase. Oxygen will decrease in concentration from the top of the tube downwards as it diffuses into the salt medium from the air above the medium. The optimal growth temperature is 17–20°C and temperatures above 25–30°C are lethal. Iron sulfide or iron carbonate can be used as a source of ferrous iron. The culture grows to a maximum of 5×10^6 cells/ml culture (Fig. BXII.β.101C), which makes many traditional techniques for strain characterization impossible. Strain BD develops small, circular, colonies attached to the wall of the tube, 3–5 d after inoculation. Strain Johan predominantly forms a ring at a specific level in the concentration gradient. The cells of strain Johan colonize as new rings from upper levels in the tube as the culture gets old, i.e., 10 days or more. Eventually, most of the tube will be filled by a brittle mass of stalks, iron oxides, and cells. Hallbeck and Pedersen (1990) have demonstrated that *Gallionella* strain Johan is free living *in vitro* in its exponential growth phase and does not produce stalks until late exponential and stationary phases. The generation time at optimal temperature for strain Johan is 8.3 h. Stalk production continues for many days in stationary phase. Some cell division may still occur but not at the growth rate observed during the first 4–5 d after inoculation. The maximum mean stalk length

per cell of strain Johan has been determined to be 60 μm in a 16-day-old culture. Individual cells may produce much longer stalks; 400 μm has been reported for a single cell of strain BD. With prolonged subculturing on iron carbonate as energy source, some of the *Gallionella* strain Johan cultures were found to have irreversibly lost their ability to form a stalk (Hallbeck et al., 1993). Their identity was confirmed by 16S rRNA gene sequencing. They still form a ring of oxidized iron as the stalk-forming variant does, but the ring is very thin.

Gallionella is chemolithotrophic with ferrous iron as energy source and electron donor and with carbon dioxide as sole carbon source. CO_2 fixation by strain Johan was revealed using hydrogen [^{14}C]-carbonate (Hallbeck and Pedersen, 1991), while *in vivo* activity of the Calvin cycle key enzyme, ribulose bisphosphate-carboxylase, has been reported for strain BD (Hanert, 1989). Mixotrophic metabolism has been shown on glucose, fructose, and sucrose. Growth did not occur on these sugars without ferrous iron and carbon dioxide. Ammonium and nitrate can be used as nitrogen sources; the capacity for nitrogen fixation is unknown.

The environment where stalk-forming *Gallionella* can be found, commonly attached to surfaces, is slowly flowing groundwater that is rich in ferrous iron but has a low organic carbon content. Typical places to search for *Gallionella* are in drainpipes, storage basins for groundwater from deep wells, in tunnels, and on rock walls with seeping groundwater. A common feature of these environments is that cold (below 20°C), reduced, anaerobic, and ferrous-iron-bearing groundwater reaches an oxygen-containing atmosphere. Such environments are suitable for chemolithotrophic growth with ferrous iron as energy source and electron donor, and oxygen as electron acceptor. A slow flow, supplying ferrous iron and possibly carbonate from the groundwater to the attached cells, seems to be an absolute prerequisite for growth of *Gallionella*. In this situation, the stalk may act as a holdfast and prevent the cells from being washed out to a more oxidized environment without ferrous iron.

Hallbeck and Pedersen (1995) have demonstrated an additional function for the stalk. The iron oxidation that occurs in a typical *Gallionella* environment can be differentiated into two
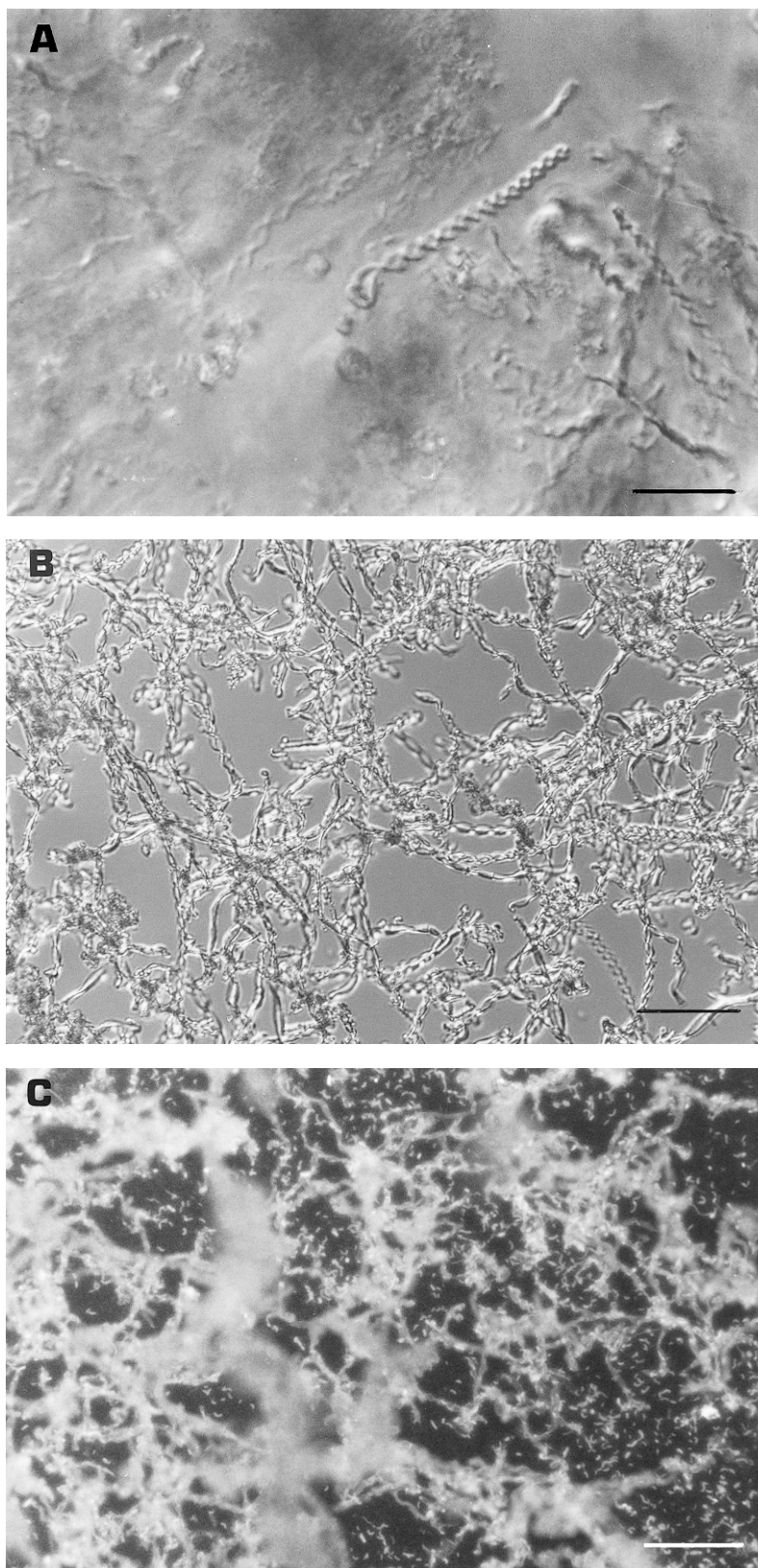
FIGURE BXII.β.101. Light microscopy images of cells and stalks of *G. ferruginea* strain Johan. *A*, Normarski microscope image of a stalked cell from an environmental sample. Bar = 10 μm. *B*, Normarski microscope image of stalks with precipitated iron from a culture. Bar = 50 μm. *C*, Fluorescent microscopy image of an acridine orange stained culture, filtered on a 0.2 μm membrane filter. Note the abundant cells among the stalks. Bar = 50 μm.
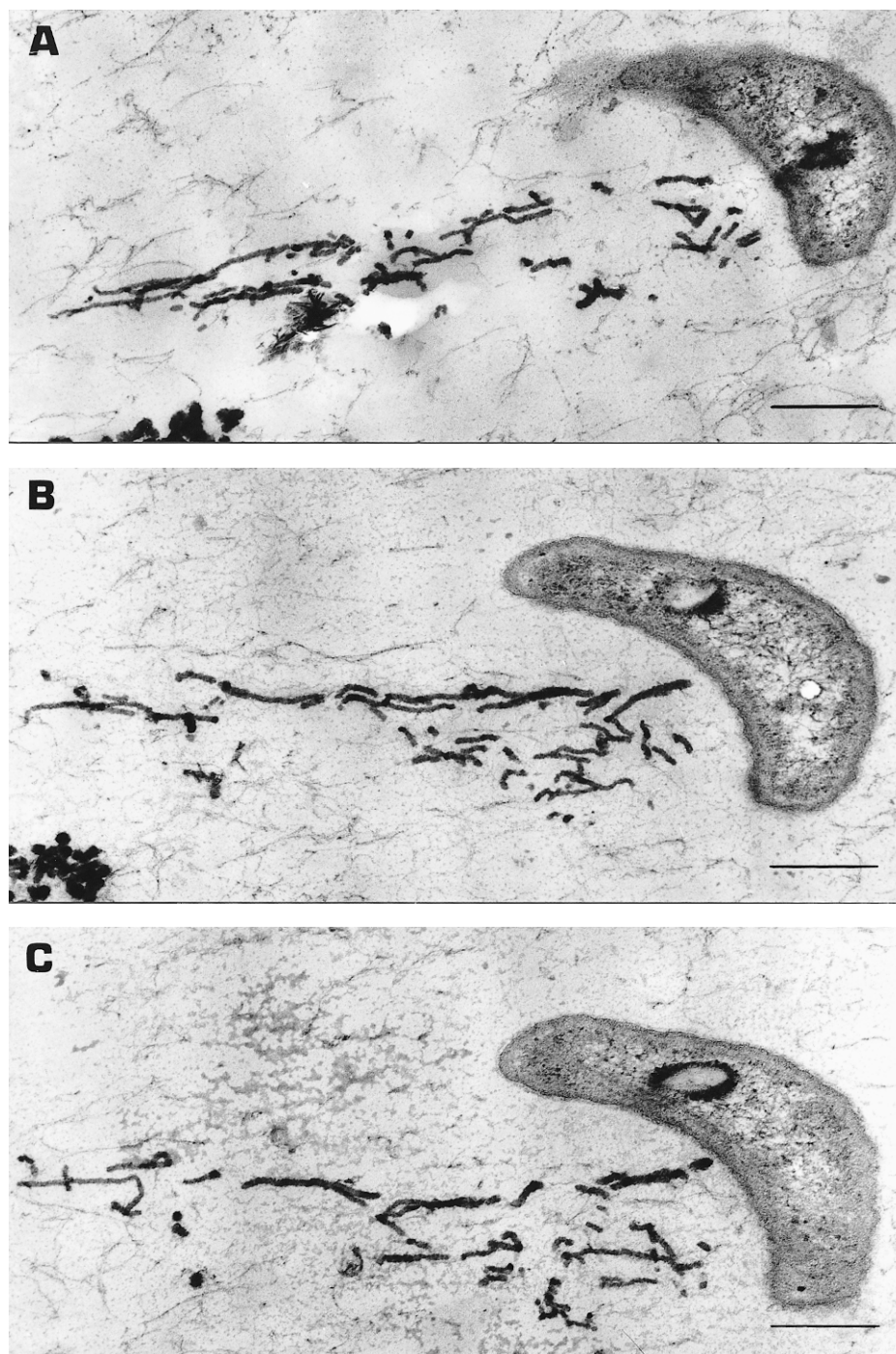
FIGURE BXII.β.102. Ultra thin section of *G. ferruginea* strain Johan in stationary phase. *A–C*, Serial thin sections of a cell with an adjacent stalk. Bars = 0.5 μm. *D*, Cross section of strain Johan with cell wall outer membrane (see next page). Bar = 0.2 μm. *E*, Longitudinal section of strain Johan. Bar = 0.25 μm (see next page).

(continued)

parts: a) The respiratory iron oxidation performed by the cells in their energy metabolism and b) the nonmetabolic iron oxidation induced by the increasing oxygen tension as the anoxic groundwater reaches the atmosphere. Ferrous iron reacting with oxygen participates in a chain of reactions yielding highly reactive oxygen such as perhydroxyl (HO_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO) (Stumm and Morgan, 1996). The survival of stalk-forming *Gallionella* strain Johan (Sta$^+$) in media

with low and high potential for oxygen radical formation was compared with a variant of strain Johan that irreversibly lost the ability to form a stalk (Sta$^-$) (Hallbeck and Pedersen, 1995). It was found that *Gallionella* Sta$^+$ survived longer (9 weeks) than Sta$^-$ (6 weeks) in cultures with a high potential for oxygen radical formation. It was therefore suggested that the stalk of *Gallionella* protects the cells against the toxic oxygen species discussed above, by directing the oxidation of iron to the stalk. This phe-
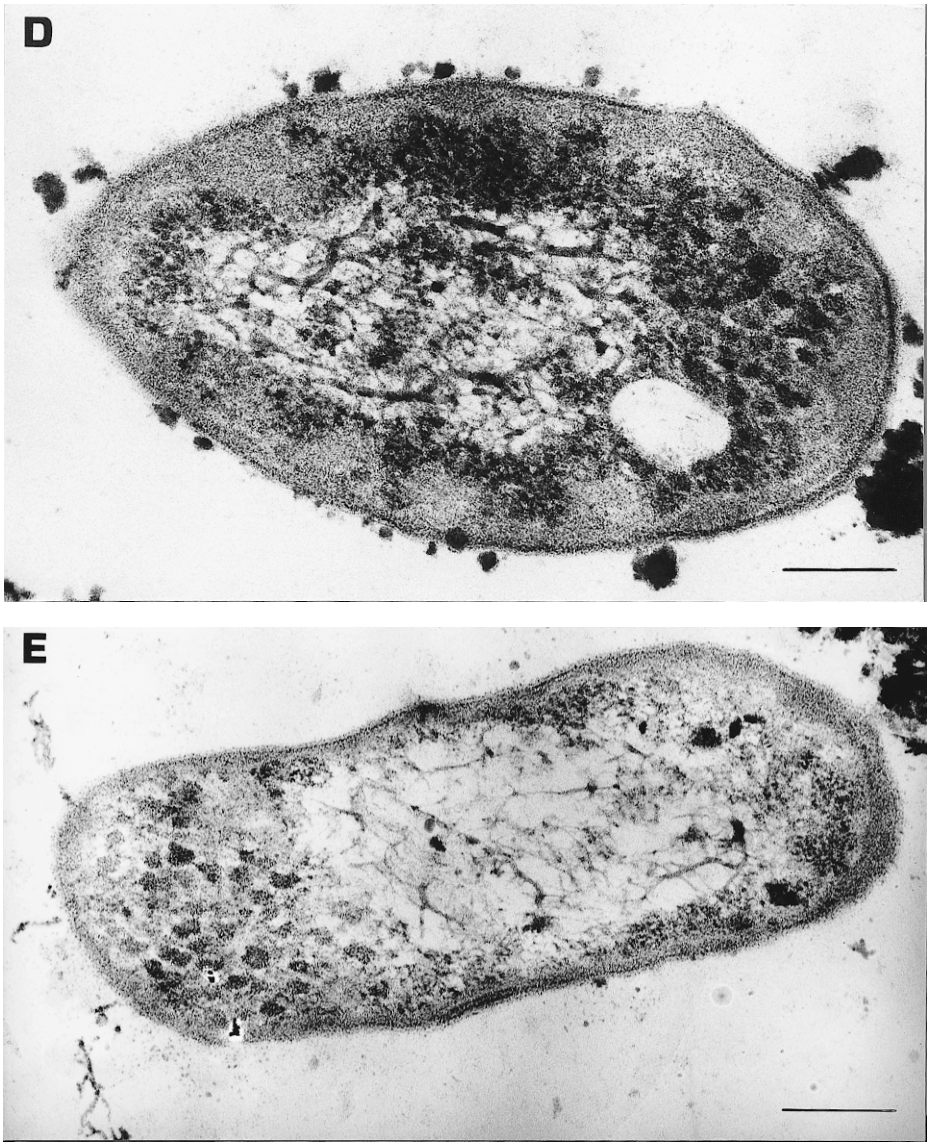
FIGURE BXII.β.102. *(continued)*

TABLE BXII.β.108. Characteristics for identification of the genus *Gallionella*

Characteristic	*Gallionella*
Cell morphology	Bean shaped
Cell dimension, μm	0.5–0.8 × 0.8–2.5
Produce an extracellular twisted stalk composed of numerous 2 nm-wide fibers	+
Grows with CO_2 as sole carbon source	+
Grows with ferrous iron as energy source	+
Temperature range (°C)	5–25
Optimal temperature (°C)	17–20
pH range	5.0–6.5
Motile	+

nomenon could be compared to the action of the protein ferritin, proposed to perform iron oxidation in both procaryotic and eucaryotic cells (Artymiuk et al., 1991). It is not known whether the iron oxidation on the stalk is enzymatic or the stalk acts as a surface catalyst for the oxidation reaction. Thus, the stalk acts as a holdfast that allows *Gallionella* to colonize and survive in an ecological niche, with high ferrous iron content and some oxygen, that is unavailable for bacteria without a defense system against the oxygen radicals formed during inorganic oxidation of ferrous iron.

ENRICHMENT AND ISOLATION PROCEDURES

Various media for enrichment and cultivation of *Gallionella* have been tested. To create proper growth conditions, ferrous iron and CO_2 must be in the medium. Lieske (1911) designed a culture medium composed of carbonic water and metallic iron. The use of iron sulfide as a source of reduced iron was first suggested by Van Niel (Vatter and Wolfe, 1956). Kucera and Wolfe (1957) published a growth medium composed of a salt solution and iron sulfide, which made it possible to obtain pure cultures of *Gallionella*. The salt solution was initially prepared with tap water, because the medium lacked a crucial component. This component was later found to be calcium. Since then the ferrous iron medium has been widely used with some minor modifications.

TABLE BXII.β.109. Differential characteristics of *Gallionella ferruginea* strains Johan and BD

Characteristic	*Gallionella ferruginea* strain Johan	*Gallionella ferruginea* strain BD
Cells bean-shaped	+	+
Cell dimensions (μm)	$0.5–0.8 \times 1.6–2.5$	$0.5–0.7 \times 0.8–1.8$
Temperature range for growth (°C)	5–25	nd
Optimal temperature (°C)	20	17
pH range for exponential growth	5.0–6.5	nd
Generation time in exponential growth	8.3 h	nd
Motility without stalks	+	+
Motility with stalks	−	−
Stalks not produced in exponential growth phases	+	nd
Length of stalks (μm)	average 60/cell	up to 400/cell
Maximum cell number in *in vitro* culture (cells/ml)	5×10^6	nd
Colony form *in vitro*	Ring on the tube wall	Circular colonies
Growth with CO_2 as sole carbon source	+	+
Growth with ferrous iron as sole energy source	+	+

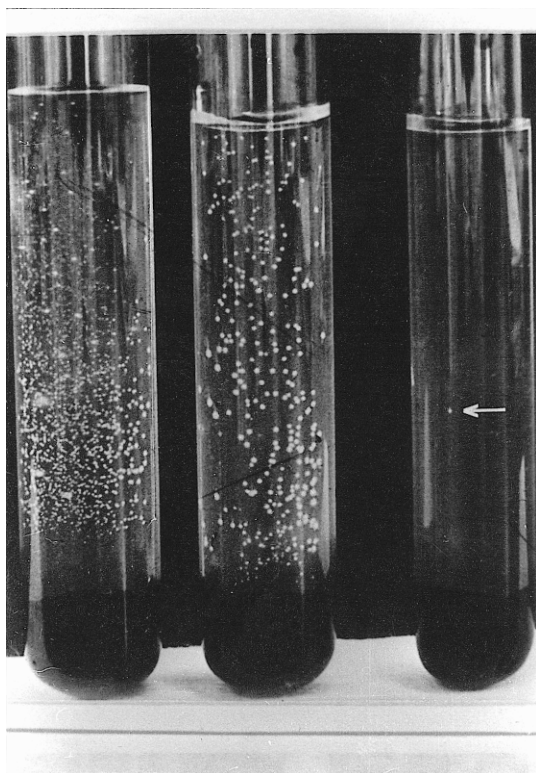[a]For symbols, see standard definitions; nd, not determined.

FIGURE BXII.β.103. Isolation procedure for *G. ferruginea* strain BD (*arrow*, one-colony culture). (Reproduced with permission from H.H. Hanert, Archives of Microbiology 60: 348–376, 1968, ©Springer-Verlag, Berlin.)

Wolfe's modified medium is made as follows: Screw-capped tubes (180 × 16 mm) are filled with 10 ml salt medium consisting of 1.0 g NH_4Cl, 0.4 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.05 g K_2HPO_4 and 1 liter double-distilled water. The salt medium is autoclaved, chilled to 5°C and infused with sterile filtered CO_2 to pH 4.6–4.8. A ferrous sulfide or ferrous carbonate precipitate (0.5 ml) is added slowly to the bottom of the tubes with a Pasteur pipette and the tubes are left for four to six hours to allow gradient conditions to establish before inoculation. The ferrous sulfide and ferrous carbonate have to be prepared in the laboratory. Ferrous sulfide is prepared by dissolving 7.8 g $FeSO_4(N_6H_4)_2SO_4$ (Mohr's salt) and 4.8 g sodium sulfide separately, each in 200 ml boiling, distilled water and subsequently pouring the ferrous solution into the sulfide solution. Use two 500 ml beakers, mix with a glass rod. Fill the beaker up to the top and seal with a rubber stopper to avoid oxidation of the ferrous iron. Let the iron sulfide sediment for at least four hours. Decant and wash with boiling water five times. Centrifuge at high velocity, collect the iron sulfide in small bottles, fill up with water and close with an airtight lid. Sterilize at 121°C for 20 min. Store cool in airtight vials. Ferrous carbonate is prepared by dissolving 3.9 g $FeSO_4(NH_4)_2SO_4$ and 1.0 g anhydrous Na_2CO_3 separately, each in 100 ml boiling distilled water, and subsequently pouring the carbonate solution into the ferrous solution, preferably under a nitrogen atmosphere. The precipitated ferrous carbonate is then washed five times with boiling double-distilled water and sterilized in closed vials at 121°C for 20 min. Store cool in airtight vials. It is recommended that all solutions used for preparation of the medium and source of ferrous iron are filter sterilized (0.2 μm) to remove any cells or particles that may give a background during microscopic counts.

Isolation by serial dilution and serial transfer has been applied successfully to *Gallionella* strains BD and Johan (Fig. BXII.β.103). For strain BD, an enrichment test tube with colonies attached to the wall of the test tube is washed several times (Hanert, 1989). One colony is subsequently suspended in 10 ml fresh sterile medium and inoculated into new test tubes with Wolfe's modified medium at dilutions of maximally 10^{-6} (30–50 parallel cultures starting with a 10^{-4} serial dilution). Five to ten serial transfers, each starting from one colony, are necessary to achieve pure cultures in this manner. This procedure requires up to 10 weeks but is a very certain method for continually reducing the number of contaminants and obtaining a pure culture. Purity is checked microscopically and by use of a variety of heterotrophic and autotrophic media i.e., yeast extract bouillon, nutrient agar, *Nitrosomonas* medium, and *Thiobacillus ferrooxidans* medium. Stalk material of strain Johan can be collected, suspended in new medium, diluted and inoculated according to the strain BD procedure.

MAINTENANCE PROCEDURES

Maintaining pure stock cultures of *Gallionella* strain BD and Johan over the past years was performed using the described culture conditions with serial dilution transfers every four to eight weeks. Preservation of *Gallionella* culture material for at least 13 weeks by freezing at −80°C in 15% glycerol has been reported

by Nunley and Krieg (1968). This procedure, however, did not result in survival of strain Johan.

TAXONOMIC COMMENTS

The genus *Gallionella* is characterized by its chemolithotrophic growth with ferrous iron, and its production of a twisted stalk consisting of a bundle of numerous fibers that makes *Gallionella* very easy to identify. *Gallionella* has been described under several different names such as *Spirophyllum ferrugineum* (Ehrenberg, 1836; Adler, 1904), *Didymohelix ferrugineum* (Griffith, 1853), *Gloeosphaera ferruginea* (Rabenhorst, 1854), and *Gallionella filamenta* (Balashova, 1967a, b). Most, if not all, of the attempts to categorize species of the genus *Gallionella* under various names arise from observed differences in the appearance of the stalk. The current phylogenetic position of the family *Gallionellaceae* is based on only a single 16S rRNA gene sequence of *Gallionella*.

More than 160 years ago, in 1836, Ehrenberg first discovered the stalks of *Gallionella* when he studied ochre masses. In this description (Ehrenberg, 1836), *Gallionella* was referred to as a fossil infusorian and he called it "die Eisenochertierchen", the small iron ochre animal. Haeckel (1866) presented a phylogenetic tree that for the first time included the kingdom *Monera* for unicellular organisms and Zopf (1879) first included *Gallionella* with the *Bacteria*. *Gallionella* has been observed and described by many more scientists since these early days, and there has been continuous discussion about its morphology and physiology, but most studies have focused on the stalks. Beger and Bringmann (1953) and van Iterson (1958) have summarized the first 100 years of discussion about the intriguing characteristics of *Gallionella*. Winogradsky (1888, 1922) proposed an autolithotrophic life for the so-called "iron bacteria", including *Gallionella*. He mentioned *Leptothrix*, *Cladothrix*, and *Gallionella* as examples of lithotrophic iron bacteria. Adler (1904) found only small amounts of *Gallionella* in fresh water from Karlspader, but when the water was left in bottles for several days they "ausserordentlich stark vermehrt" i.e., they had grown, or more correctly, the stalks had become elongated. Lieske (1911) succeeded in the cultivation of *Gallionella* in carbonic water with metallic iron as ferrous iron source. In 1924–1929, Cholodny made microscopic studies on cover slips that he had submerged in habitats of *Gallionella* (Cholodny, 1924, 1929). He sketched admirable pictures of cells attached to the end of stalks, and he showed that the stalk was excreted by the cell and was not a living part of it. Teichmann (1935) made cultures according to Lieske (1911) and found a great number of bean-shaped cells in the fluid. This observation influenced Pringsheim (1949b) to suggest

that "It is not impossible that motile cells are formed under certain conditions", a conclusion in accordance with current knowledge of free-living cells in exponential growth phase. Beger and Bringmann (1953) made comparisons between earlier drawings of the stalk of *Gallionella* and their own electron microscopy studies and proposed that the genus *Gallionella* consisted of five species. Vatter and Wolfe (1956) presented electron microscopy images of cells with stalks and in 1957, Kucera and Wolfe (1957) introduced an excellent growth medium containing iron sulfide as the source of ferrous iron. Wolfe could have succeeded in working with *Gallionella* but he concluded that "these organisms (iron bacteria) are too difficult to be profitable" (Wolfe, 1964). In 1958, a thesis on *Gallionella* was presented by van Iterson (1958). Excellent electron microscopy images of the organism were presented and it was suggested that the stalk was a living part of the organism with sporangia in the form of membrane sacs on the stalk. Balashova (1967a,b, 1968) and Balashova and Cherni (1970) made electron microscopy observations of the stalk and concluded that the stalk might have zoogloeal forms and budding cells on the stalks. Hanert (1989) made detailed studies on stalk elongation using single cells and measurements of iron oxidation in both natural samples and lab cultures. Intracytoplasmic membranes and evidence for autotrophic growth was presented. In 1990, Lütters-Czekalla (1990) reported growth of *Gallionella* BD with reduced sulfur compounds as electron donor instead of ferrous iron. This observation remains to be confirmed. In 1990–1995 Hallbeck and Pedersen (1990, 1991, 1995) and Hallbeck et al. (1993) reported the 16S rDNA sequence of *Gallionella* and showed that *Gallionella* has a free-living stage without a stalk in exponential growth phase. They demonstrated chemolithotrophic growth with CO_2 as the sole carbon source and mixotrophic metabolism. They also demonstrated an organic composition of the stalk and found that the stalk was important for survival in the environments where *Gallionella* is found. A thesis summarizing these results was published in 1993 (see further reading).

FURTHER READING

Hallbeck, L. 1993. On the biology of the iron-oxidizing and stalk-forming bacterium *Gallionella ferruginea*, Thesis, Göteborg University, Göteborg Sweden.

List of species of the genus *Gallionella*

1. **Gallionella ferruginea** Ehrenberg 1838, 166[AL]

   fer.ru.gi′ ne.a. L. fem. adj. *ferruginea* rust-colored.

   Morphology and description the same as those of the genus. Organisms occur in groundwater habitats, especially in places where iron-bearing groundwater reaches an oxygen-containing environment. Microaerophilic, possibly fac-

ultatively anaerobic, chemolithotrophic, and mixotrophic. Gram negative. G + C buoyant density was calculated by using a micromethod with novel collimating optics (Hanert 1989). (*E. coli* B was used as a reference.)

The mol% G + C of the DNA is: 51–54.6. (Bd).

Type strain: no culture isolated.

GenBank accession number (16S rRNA): L07897.

Order VI. **Rhodocyclales** *ord. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Rho.do.cy.cla' les. M.L. masc. n. *Rhodocyclus* type genus of the order; *-ales* suffix to denote
order; M.L. fem. n. *Rhodocyclales* the *Rhodocyclus* order.

The order *Rhodocyclales* was circumscribed for this volume on the
basis of phylogenetic analysis of 16S rRNA sequences; the order
contains the family *Rhodocyclaceae*.

Description is the same as for the family *Rhodocyclaceae*.
Type genus: **Rhodocyclus** Pfennig 1978, 285.

Family I. **Rhodocyclaceae** *fam. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Rho.do.cy.cla' ce.ae. M.L. masc. n. *Rhodocyclus* type genus of the family; *-aceae* ending to
denote family; M.L. fem. pl. n. *Rhodocyclaceae* the *Rhodocyclus* family.

The family *Rhodocyclaceae* was circumscribed for this volume on
the basis of phylogenetic analysis of 16S rRNA sequences; the
family contains the genera *Rhodocyclus* (type genus), *Azoarcus,
Azonexus, Azospira, Azovibrio, Dechloromonas, Dechlorosoma, Ferribac-
terium, Propionibacter, Propionivibrio, Quadricoccus, Sterolibacterium,
Thauera,* and *Zoogloea. Quadricoccus* and *Sterolibacterium* were pro-
posed after the cut-off date for inclusion in this volume (June

30, 2001) and are not described here (see Maszenan et al., 2002,
and Tarlera and Denner, 2003).

Family is phenotypically, metabolically, and ecologically di-
verse. Includes photoheterotrophs; aerobes, anaerobes, and fac-
ultative anaerobes utilizing a number of electron acceptors; fer-
mentative organisms; and nitrogen-fixing organisms.
Type genus: **Rhodocyclus** Pfennig 1978, 285.

Genus I. **Rhodocyclus** *Pfennig 1978, 285*[AL]

JOHANNES F. IMHOFF

Rho.do.cy' clus. Gr. n. *rhodon* the rose; Gr. n. *cyclos* a circle; M.L. masc. n. *Rhodocyclus* red circle.

Cells are slender, curved, or straight, thin rods. Motile by means
of polar flagella or nonmotile. Multiply by binary fission. **Gram
negative, belonging to class *Betaproteobacteria*.** Internal photo-
synthetic membranes may appear as small, single, finger-like in-
trusions of the cytoplasmic membrane or may be absent. **Pho-
tosynthetic pigments are bacteriochlorophyll *a* and carotenoids.
Contain ubiquinones and menaquinones with eight isoprene units
(Q-8 and MK-8). Straight-chain $C_{16:1}$ and $C_{16:0}$ acids are the major
components of cellular fatty acids. $C_{10:0\ 3OH}$ is present.**

Preferably grow photoheterotrophically under anoxic condi-
tions in the light with different organic substrates as carbon and
electron sources. **Photoautotrophic growth with molecular hy-
drogen** may be possible if growth factors are supplied. **Chemo-
trophic growth is also possible under microoxic to oxic conditions
in the dark.** Reduced sulfur compounds are not used as photo-
synthetic electron donors. Assimilatory sulfate reduction is pos-
sible. Growth factors may be required. **Mesophilic and neutro-
philic freshwater bacteria.** Habitat: freshwater ponds, sewage
ditches, swine waste lagoon.

The mol% G + C of the DNA is: 64.1–65.1.
Type species: **Rhodocyclus purpureus** Pfennig 1978, 285.

FURTHER DESCRIPTIVE INFORMATION

Only a few carbon compounds can be assimilated by *Rhodocyclus
purpureus.* Benzoate and cyclohexane carboxylate are both used,
which may indicate that *Rhodocyclus purpureus* uses the same path-
way for anaerobic benzoate degradation as *Rhodopseudomonas pa-
lustris* (Dutton and Evans, 1969; Pfennig, 1978). Neither of these
carbon substrates is used by other *Rhodocyclus* species, and they
are used only rarely by other purple nonsulfur bacteria.

Nitrogen metabolism of *R. purpureus* and *R. tenuis* has been

studied in some detail (Masters and Madigan, 1983). Alanine
dehydrogenase is absent in both species. Glutamate dehydro-
genase (NADPH-dependent) is found in *R. purpureus* at unusually
high activity levels under all growth conditions, and the gluta-
mine synthetase inhibitor methionine sulfoximine exerts no
growth inhibition. This may indicate that the major route of
nitrogen assimilation in *R. purpureus* is via glutamate dehydro-
genase (unlike that in all other investigated purple nonsulfur
bacteria). *R. tenuis* employs the glutamine synthetase/glutamate
synthase (NADPH-dependent) pathway for the assimilation of
ammonia (Masters and Madigan, 1983).

As with other phototrophic *Betaproteobacteria*, the major phos-
pholipid components of *Rhodocyclus* species are cardiolipin, phos-
phatidylethanolamine and phosphatidylglycerol (Imhoff and
Bias-Imhoff, 1995). Straight-chain $C_{16:1}$ and $C_{16:0}$ acids are the
main components of cellular fatty acids (see Table BXII.β.110).

ENRICHMENT AND ISOLATION PROCEDURES

R. purpureus was isolated from a swine waste lagoon in Ames,
Iowa (USA), where it was the dominant phototrophic bacterium.
It has not been observed in other localities and is probably a
rare species. Media for enrichment, isolation and growth of *Rho-
docyclus* species are the same as those generally employed for
other purple nonsulfur bacteria. While suitable conditions for
selective enrichment of *R. tenuis* are not available, the vitamin
B_{12} requirement and its unusual carbon nutrition are properties
that can be exploited for the selective enrichment and isolation
of *R. purpureus*. From a suitable habitat, it should be possible to
selectively enrich this species with benzoic acid as the carbon
source, in the presence of vitamin B_{12}, and in the absence of
reduced sulfur compounds.

TABLE BXII.β.110. Differential characteristics of the species of the genus *Rhodocyclus*[a]

Characteristic	*Rhodocyclus purpureus*	*Rhodocyclus tenuis*
Cell diameter (μm)	0.6–0.7	0.3–0.5
Cell shape	Half-circle to circle	Curved rods
Motility	−	+
Slime production	−	+
Color	Purple-violet to violet	Brownish-red or purple-violet
Major carotenoids	Rhodopin, rhodopinal	Rhodopin, rhodopinal, lycopene[b]
Growth factors	B_{12}, *p*-aminobenzoic acid, biotin	None[c]
Gelatin liquefaction	−	−
Fructose fermentation	−	−
Starch hydrolysis	nd	nd
Tween 80 lysis	nd	nd
Carbon sources:		
Benzoate	+	−
C_{10} to C_{18} fatty acids	−	+
Citrate	−	−
Mannitol	−	−
Sorbitol	−	−
N_2-fixation	−	+
Fumarate reductase activity:		
With reduced methylviologen	High	High
With $FMNH_2$	Low	Low
Major fatty acids:		
$C_{16:0}$	33–35	33-3-6
$C_{16:1}$	40–45	43–50
$C_{18:0}$	<1	<1
$C_{18:1}$	18	15–18
3-OH fatty acid	$C_{10:0}$	$C_{10:0}$
Major quinones	Q-8 + MK-8	Q-8 + MK-8
Mol% G + C of DNA		
by HPLC	65.1	64.1–64.8
by Bd	65.3	64.8
by T_m	67.7	64.4–67.2

[a]Symbols: +, positive in most strains; −, negative in most strains; Q–8, ubiquinone-8; MK-8, menaquinone-8.

[b]Some strains may contain carotenoids of the spirilloxanthin series and lack rhodopinal (Schmidt, 1978).

[c]Some strains may require vitamin B_{12} (Siefert and Koppenhagen, 1982).

MAINTENANCE PROCEDURES

Rhodocyclus species are easily maintained by standard procedures in liquid nitrogen or by storage at −80°C in a mechanical freezer.

DIFFERENTIATION OF THE GENUS *RHODOCYCLUS* FROM OTHER GENERA

Rhodocyclus species are distinguished from other phototrophic purple bacteria by their separate phylogenetic position within the class *Betaproteobacteria*. Physiological properties are, in general, not significantly different from those of other purple nonsulfur bacteria, although the utilization of benzoic acid by *R. purpureus* is not a common property among other purple nonsulfur bacteria. The requirement for vitamin B_{12} is also unusual among the purple nonsulfur bacteria, being more common among the green and purple sulfur bacteria. Besides *Rhodocyclus purpureus*, only single strains of *Rhodocyclus tenuis* and *Rhodopseudomonas palustris* have been reported to require vitamin B_{12} (Siefert and Koppenhagen, 1982). Properties that differentiate *Rhodocyclus* species from other phototrophic *Betaproteobacteria* are given in Tables 6 (p. 130) and 7 (p. 131) of the introductory chapter "Anoxygenic Phototrophic Purple Bacteria", Volume 2,

Part A. The phylogenetic relationships among the phototrophic *Betaproteobacteria* are shown in Fig. 4 (p. 132) of that same chapter.

TAXONOMIC COMMENTS

At present, this genus comprises two species, *Rhodocyclus purpureus* and *Rhodocyclus tenuis* (formerly *Rhodospirillum tenue*). Only a single strain of *R. purpureus* is known. A dichotomy has been observed in *R. tenuis* strains based on carotenoid composition (Schmidt, 1978), color of cell suspensions, and absorption spectra (Biebl, 1973). Some strains of *R. tenuis* have carotenoids of the rhodopinal series and others have carotenoids of the spirilloxanthin series. The type strain (DSM 109) belongs to those strains that transform rhodopin further to spirilloxanthin, whereas another group of strains (including strain DSM 110) does not form anhydrorhodovibrin and spirilloxanthin, but rather accumulates major amounts of rhodopinal, rhodopinol, and lycopenal (Schmidt, 1978). Both of these strains (DSM 109 and 110) show a reasonably close genetic relationship to each other (see Fig. 4 [p.132] of the introductory chapter "Anoxygenic Phototrophic Purple Bacteria", Volume 2, Part A).

DIFFERENTIATION OF THE SPECIES OF THE GENUS *RHODOCYCLUS*

While cells of *Rhodocyclus purpureus* are nonmotile and form a half or full circle, those of *Rhodocyclus tenuis* are slender, slightly

curved, and rapidly motile under optimal growth conditions. *R. purpureus* and *R. tenuis* also differ significantly in their nitrogen

nutrition. Whereas the former species uses only ammonia and glutamine as nitrogen sources and is unable to fix dinitrogen (a property common to most species of the purple nonsulfur bacteria), the latter species utilizes a greater number of amino acids, urea, dinitrogen, yeast extract, peptone, and Casamino acids (Masters and Madigan, 1983). In addition, differences in carbon nutrition and vitamin requirements clearly differentiate the two species. Diagnostic properties of *Rhodocyclus* species are listed in Table BXII.β.110. Their carbon substrates are shown in Table BXII.β.111.

List of species of the genus Rhodocyclus

1. **Rhodocyclus purpureus** Pfennig 1978, 285[AL]

   pur.pu′ re.us. L. adj. *purpureus* purple or red-violet.

   Cells are half-ring-shaped to ring-shaped before cell division and are 0.6–0.7 μm wide. The diameter of a circle is 2.0–3.0 μm. Half-circle-shaped cells are about 2.7 μm long (Fig. BXII.β.104). Open or compact spirals or coils of variable length may be formed. In sulfide-containing media, closely wound spirals are united in compact cell aggregates. Cells are nonmotile under all growth conditions. The color of phototrophically grown cultures is purple-violet to violet. Aerobically grown cells are colorless to pale violet. Living cells have absorption maxima at 379, 408, 510, 535, 597, 813, and 866 nm. Photosynthetic pigments are bacteriochlorophyll *a* (esterified with phytol) and carotenoids of the rhodopinal series, with rhodopinal as the major component.

   Photoheterotrophic growth occurs under anoxic conditions in the light with a relatively small number of organic substrates as carbon and electron sources. Cells grow photoautotrophically with hydrogen as the electron donor in the presence of growth factors. Chemotrophic growth is possible under microoxic to oxic conditions in the dark. Carbon sources utilized are listed in Table BXII.β.111. In addition, cyclohexane carboxylate is used, but propanol, yeast extract, and Casamino acids are not utilized as sole carbon sources. Photoheterotrophically grown cells use only ammonia and glutamine as nitrogen sources; dinitrogen is not assimilated. Sulfate can be used as the sole sulfur source. Vitamin B_{12}, *p*-aminobenzoic acid, and biotin are required as growth factors.

   Mesophilic freshwater bacterium with optimal growth at 30°C and pH 7.2 (pH range with acetate: pH 6.5–7.5). Habitat: swine waste lagoon. Major quinone components are Q-8 and MK-8.

   The mol% G + C of the DNA is: 65.3 (Bd), 65.1 (HPLC), 67.7 (T_m).

   Type strain: Ames 6770, DSM 168.

   GenBank accession number (16S rRNA): M34132.

2. **Rhodocyclus tenuis** (Pfennig 1969b) Imhoff, Trüper and Pfennig 1984, 341[VP] (*Rhodospirillum tenue* Pfennig 1969b, 619.)

   te′ nu.is. L. masc. adj. *tenuis* slender, thin.

   Cells are curved in spirals of one to two complete turns, 0.3–0.5 × 1.5–6.0 μm, sometimes even longer. One complete turn of a spiral is ~0.8–1.0 μm wide and 3 μm long (Fig. BXII.β.105). Photosynthetically grown cells are brownish-red or purple-violet, depending on the strain. Aerobically grown cells may be colorless or pigmented. Absorption maxima of brownish cells that have carotenoids of the spi-

TABLE BXII.β.111. Carbon sources and electron donors used by the species of the genera *Rhodocyclus*[a]

Source/donor	*Rhodocyclus purpureus*	*Rhodocyclus tenuis*
Carbon source		
Acetate	+	+
Arginine	−	−
Aspartate	−	−
Benzoate	+	−
Butyrate	+	+
Caproate	+	+
Caprylate	−	+/−
Citrate	−	−
Ethanol	−	+/−
Formate	−	−
Fructose	−	−
Fumarate	+	+
Glucose	−	−
Glutamate	−	−
Glycerol	−	−
Glycolate	−	−
Lactate	−	+
Malate	+	+
Malonate	−	−
Mannitol	−	−
Mannose	−-	−
Methanol	−	−
Pelargonate	−	+
Propionate	−	+/−
Pyruvate	+	+
Sorbitol	−	nd
Succinate	−	+
Tartrate	−	−
Valerate	−	+
Electron donor:		
Hydrogen	+	+
Sulfide	−	−
Sulfur	−	−
Thiosulfate	−	−

[a] Symbols: +, positive in most strains; −, negative in most strains; +/− variable in different strains; nd, not determined.
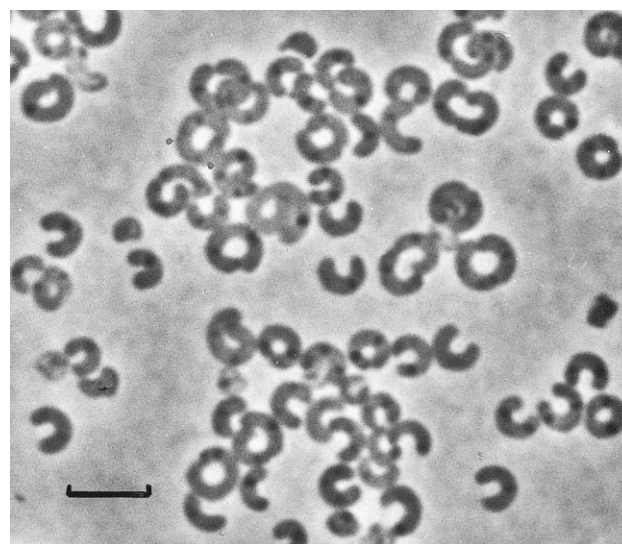

FIGURE BXII.β.104. *Rhodocyclus purpureus* DSM 168. Phase-contrast micrograph. Bar = 5 μm (Courtesy of N. Pfennig).
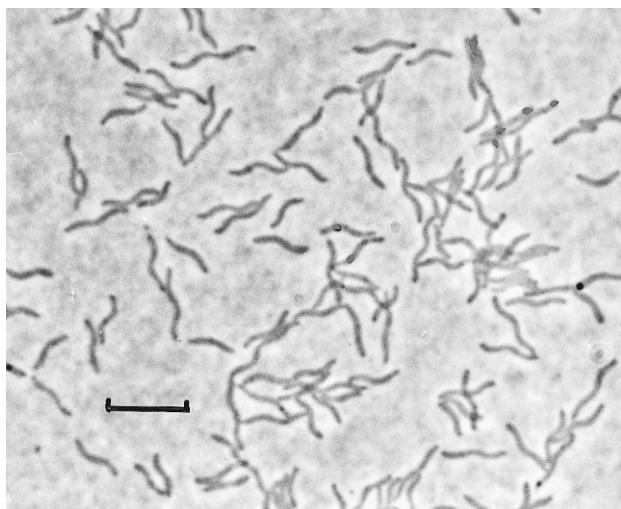
FIGURE BXII.β.105 *Rhodocyclus tenuis* DSM 109. Phase-contrast micrograph. Bar = 5 μm (Courtesy of N. Pfennig).

rilloxanthin series are at 378–380, 465, 492–495, 528, 592–594, 799–801, and 868–871 nm. Absorption maxima of purple cells that have carotenoids of the rhodopinal series are at 377–378, 469, 495–500, 529–533, 590–592, 798–801, and

856–858 nm. Both types of cells contain bacteriochlorophyll *a* esterified with phytol.

Growth occurs preferably under anoxic conditions in the light with numerous carbon substrates as carbon and electron sources. Photoautotrophic growth with molecular hydrogen is possible. Chemotrophic growth is possible under microoxic to oxic conditions in the dark. The organic substrates used are listed in Table BXII.β.111. In addition, Casamino acids and yeast extract are utilized, but cyclohexane carboxylate is not. Aspartate, glutamate, glutamine, ammonia, and dinitrogen are used as nitrogen sources; also utilized are Casamino acids, peptone, yeast extract, alanine, arginine, lysine, methionine, serine, threonine, and urea. Sulfate, sulfite, sulfide, thiosulfate, cysteine, and reduced glutathione can serve as assimilatory sulfur sources. Growth factors are not required. Growth is stimulated, however, in the presence of complex organic nutrients or yeast extract, and some strains may need vitamin B_{12}.

Mesophilic freshwater bacterium with optimal growth at 30°C and pH 6.6–7.4. Habitat: freshwater ponds, sewage ditches. Major quinone components are Q-8 and MK-8.

The mol% G + C of the DNA is: 64.1–64.8 (HPLC), 64.4–67.2 (T_m); type strain: 64.8 (Bd) and 66.1 (T_m).

Type strain: ATCC 25093, DSM 109.

GenBank accession number (16S rRNA): D16208.

Genus II. **Azoarcus** *Reinhold-Hurek, Hurek, Gillis, Hoste, Vancanneyt, Kersters and De Ley 1993b, 582^{VP}*

BARBARA REINHOLD-HUREK, ZHIYUAN TAN AND THOMAS HUREK

A.zo' ar.cus. Fr. n. *azote* nitrogen; L. masc. n. *arcus* arch, bow; M.L. masc. n. *Azoarcus* nitrogen (-fixing) bow.

Straight to slightly curved rods, 0.4–1.5 × 1.1–4.0 μm. Cell pairs often appear slightly S-shaped. In most species, some elongated cells (8–12 μm) occur in late-log or stationary-phase cultures on medium containing malic acid and N_2 or ammonium as nitrogen source. **Cells are highly motile by means of one polar flagellum. Accumulate poly-β-hydroxybutyrate granules.** Gram negative. **Some species are nitrogen fixers;** these require microaerobic conditions for growth on N_2. On semisolid nitrogen-free media, microaerophilic growth can be observed as veil-like pellicles developing several mm below the surface and moving to the medium surface during growth. In most species, colonies on VM agar[1] supplemented with ethanol develop **a nondiffusible yellowish pigment**. Optimal temperature for growth 30–40°C; no growth occurs at 45°C. **Chemoorganoheterotrophic. Bacteria have a strictly respiratory metabolism with O_2 as the terminal electron acceptor,** except one species. Alternatively, under anaerobic conditions, nitrate can be used for dissimilatory nitrate reduction. **Oxidase positive. Grow well on salts of organic acids** such as L-malate, succinate, fumarate, DL-lactate; also grow well on ethanol, on L-glutamate, but not on mono- or disaccharides except for species that are not plant-associated. These soil-borne species utilize a variety of **aromatic substrates as sole carbon sources under denitrifying conditions**. Nitrate can be used as a

nitrogen source (assimilatory nitrate reduction). Growth factor requirements vary: some strains depend on *p*-aminobenzoic acid or on cobalamine. All investigated species have $C_{16:1}$ cellular fatty acids; all species except one have $C_{16:1\ \omega7c}$ and $C_{18:1}$ as the major cellular fatty acids.

The mol% G + C of the DNA is: 62–68 (T_m).

Type species: **Azoarcus indigens** Reinhold-Hurek, Hurek, Gillis, Hoste, Vancanneyt, Kersters and De Ley 1993b, 583.

FURTHER DESCRIPTIVE INFORMATION

The members of *Azoarcus* comprise two biogroups: (1) the soil-borne species *A. tolulyticus, A. toluclasticus, A. toluvorans, A. evansii*, and *A. anaerobius*, and (2) the plant-associated species *A. indigens, A. communis*, and an unnamed *Azoarcus* strain, strain BH72 (Fig. BXII.β.106).

Morphology Cells are straight to slightly curved rods that are highly motile by one, rarely two, polar flagella (Fig. BXII.β.107). All species have a cell width of ≤1 μm, with the exception of *Azoarcus anaerobius*, which has a cell width of 1.5 μm (Springer et al., 1998). The cell length ranges from 1.5–4.0 μm. Elongated cells of 8–12 μm length are rarely found in the plant-associated species (*A. communis, A. indigens*, and *Azoarcus* strain BH72) in stationary phase cultures grown in semisolid N-free malate medium.

Growth The optimal growth temperature for most species is 30°C or 37–40°C depending on species, but is lower (28°C) for *A. anaerobius*. The optimal pH is 7.

---

1. VM agar contains (g per liter): K_2HPO_4, 0.6; KH_2PO_4, 0.4; NH_4Cl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 1.1; $CaCl_2 \cdot 2H_2O$, 0.026; $MnSO_4 \cdot H_2O$, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.002; Fe (III)-EDTA, 0.066; yeast extract, 1; Bacto-Tryptone, 3; pH 6.8; ethanol, 6 ml per l, sterilized by filtration.

Metabolism The optimal growth temperature for most species is 30°C or 37–40°C depending on species, but is lower (28°C) for *A. anaerobius*. The optimal pH is 7.

All species except *A. anaerobius* use oxygen as the terminal electron acceptor. In the original description of *Azoarcus*, standard procedures had failed to reveal an ability to denitrify (Reinhold-Hurek et al., 1993b). On closer examination, the ability to use nitrate as the terminal electron acceptor was demonstrated for the plant-associated strains (Hurek and Reinhold-Hurek, 1995), a feature that they share with all other species (Anders et al., 1995; Zhou et al., 1995; Springer et al., 1998; Song et al., 1999). Most strains belonging to *A. tolulyticus*, *A. evansii*, *A. toluclasticus*, *A. toluvorans*, and *A. anaerobius* were enriched or isolated anaerobically on nitrate, whereas the plant-associated species were enriched under nitrogen-fixing conditions.

In general, carbohydrates are not the preferred carbon sources of *Azoarcus* species. None of the plant-associated species is able to utilize any of the 50 mono- and disaccharides or sugar alcohols tested (Reinhold-Hurek et al., 1993b). Similarly, *A. anaerobius* shows no growth on common carbohydrates such as $D(+)$-glucose or $D(-)$-fructose (Springer et al., 1998). In contrast, all strains of soil-borne species tested so far are able to utilize at least some carbohydrates (see Table BXII.β.112). All strains grow well on organic acids and a few amino acids (Table BXII.β.112). The carbon sources listed in Table BXII.β.112 were tested with O_2 as the terminal electron acceptor (except in the case of *A. anaerobius*). For some strains, tests were also carried out under anaerobic conditions with nitrate as electron acceptor. For the majority of carbon sources, results were identical; however, for several carbon sources discrepancies were found between aerobic and anaerobic conditions as well as between strains (Song et al., 1999). Most soil-borne species grow on aromatic compounds such as toluene or phenol (Zhou et al., 1995; Song et al., 1999), benzoate (Anders et al., 1995), or resorcinol (Springer et al., 1998) under denitrifying conditions, in contrast to plant-associated species (Reinhold-Hurek et al., 1993b; Hurek and Reinhold-Hurek, 1995). Due to the anaerobic degradation of aromatic compounds, this bacterial group has received particular attention for its role in biodegradation and biotransformation. Whereas aerobic metabolism is characterized by the extensive use of molecular oxygen, which is essential for the hydroxylation and cleavage of the ring structures, anaerobic degradation uses other strategies that are currently being studied. Toluene, which can be decomposed anaerobically by three species, is activated by the addition of fumarate to form benzylsuccinate (Beller and Spormann, 1997). The reaction is catalyzed by benzylsuccinate synthase and involves a glycyl radical (Krieger et al., 2001). See the description of *A. evansii* for further details on degradation of aromatic compounds.

In the original description of the genus *Azoarcus*, the ability to fix nitrogen is listed as a genus character (Reinhold-Hurek et al., 1993b). Subsequently, nitrogen fixation or the occurrence of a nitrogenase gene *nifH* was also demonstrated for *A. tolulyticus* (Fries et al., 1994; Zhou et al., 1995; Hurek et al., 1997a). However, nitrogen fixation can no longer be considered a universal characteristic of the genus *Azoarcus*, due to the addition of new species that do not fix nitrogen. Nitrogen fixation was not detected in *A. anaerobius* (Springer et al., 1998). Although there are no physiological data available (Anders et al., 1995), *A. evansii* is unlikely to be diazotrophic since there is no evidence for occurrence of a PCR-amplifiable *nifH* fragment (Hurek and Reinhold-Hurek, unpublished observations). For most other isolates enriched under denitrifying conditions, this character was not tested at all.

In those species and strains that carry out nitrogen fixation (*A. indigens*, *A. communis*, *A. tolulyticus*, *Azoarcus* sp. strain BH72), nitrogenase activity occurs only under microaerobic conditions, probably due to a lack of efficient oxygen protection mechanisms. When cultured on N-free semisolid medium, the nitrogen-fixing strains develop a veil-like pellicle, which, due to aerotaxis, moves to the medium surface when the culture becomes denser. For strain BH72, nitrogen fixation is more tolerant to oxygen than in *Azospirillum* spp., reaching steady states in an oxygen-controlled chemostat up to 25 µM dissolved O_2 (Hurek et al., 1987). In accordance with these physiological data, the expression of nitrogenase genes is transcriptionally regulated in response to oxygen (fully repressed by 4% oxygen in the headspace) or combined nitrogen (repressed by 0.5 mM ammonium or nitrate) (Egener et al., 1999). In empirically optimized batch cultures, strain BH72 shows augmented rates and efficiency of nitrogen fixation, called hyperinduction, when shifting into extremely low oxygen concentrations (30 nM) (Hurek et al., 1994a). In the course of hyperinduction, novel intracytoplasmic membrane stacks are formed that might participate in efficient nitrogenase activity, since the iron protein of nitrogenase is mainly associated with these membranes (Hurek and Reinhold-Hurek, 1995). The formation of these so-called diazosomes is most abundant and reproducible in coculture of strain BH72 and an endophytic fungus isolated from Kallar grass (Hurek and Reinhold-Hurek, 1995) related to *Acremonium alternatum* (Hurek and Reinhold-Hurek, 1999). As in many *Proteobacteria*, the nitrogenase genes are organized in a *nifHDK* operon (Egener et al., 2001). Phylogenetically, the nitrogenase in the genus *Azoarcus* either follows the organismal phylogenetic tree or appears to have been acquired by lateral gene transfer, depending on the species. In *A. indigens*, *A. communis*, and *Azoarcus* spp. strain BH72, a fragment of the iron protein of nitrogenase encoded by the *nifH* gene is most closely related to nitrogenases that occur in diazotrophs of *Gammaproteobacteria*, whereas in *A. tolulyticus* it is located within a clade of nitrogenases that occur in species of *Alphaproteobacteria* (*Bradyrhizobium* and *Azospirillum*) (Hurek et al., 1997a).

Exoenzymes There are few studies of the exoenzymes of *Azoarcus* sp. In all three plant-associated species, an exoglucanase having β-glucosidase and cellobiohydrolase activity was detected, whereas an endoglucanase was found in strain BH72 and in *A. communis* only (Reinhold-Hurek et al., 1993a). Although these enzymes cannot be used for direct metabolic purposes because these strains do not grow on carbohydrates, they may be involved in plant infection.

Antigens Polyclonal antibodies have been raised against *Azoarcus* sp. strain BH72 and mainly bind to the cell surface. They cross-react weakly with *A. indigens* and *A. communis* cells. These antibodies have been used for histochemical detection of *Azoarcus* in grass roots (Hurek et al., 1994b).

Genetics DNA reassociation experiments with the three plant-associated species of *Azoarcus* have led to the estimation of a genome size from 4.5–5 Mb. Nothing is known about bacteriophages of the genus *Azoarcus*. Similarly, the plasmid content has not been well studied. In strain BH72, plasmids could not be detected in Eckhardt gels or by pulsed-field gel electrophoresis (Reinhold-Hurek and Hurek, unpublished observations). Several strains of *Azoarcus* species have been shown to be transformable.
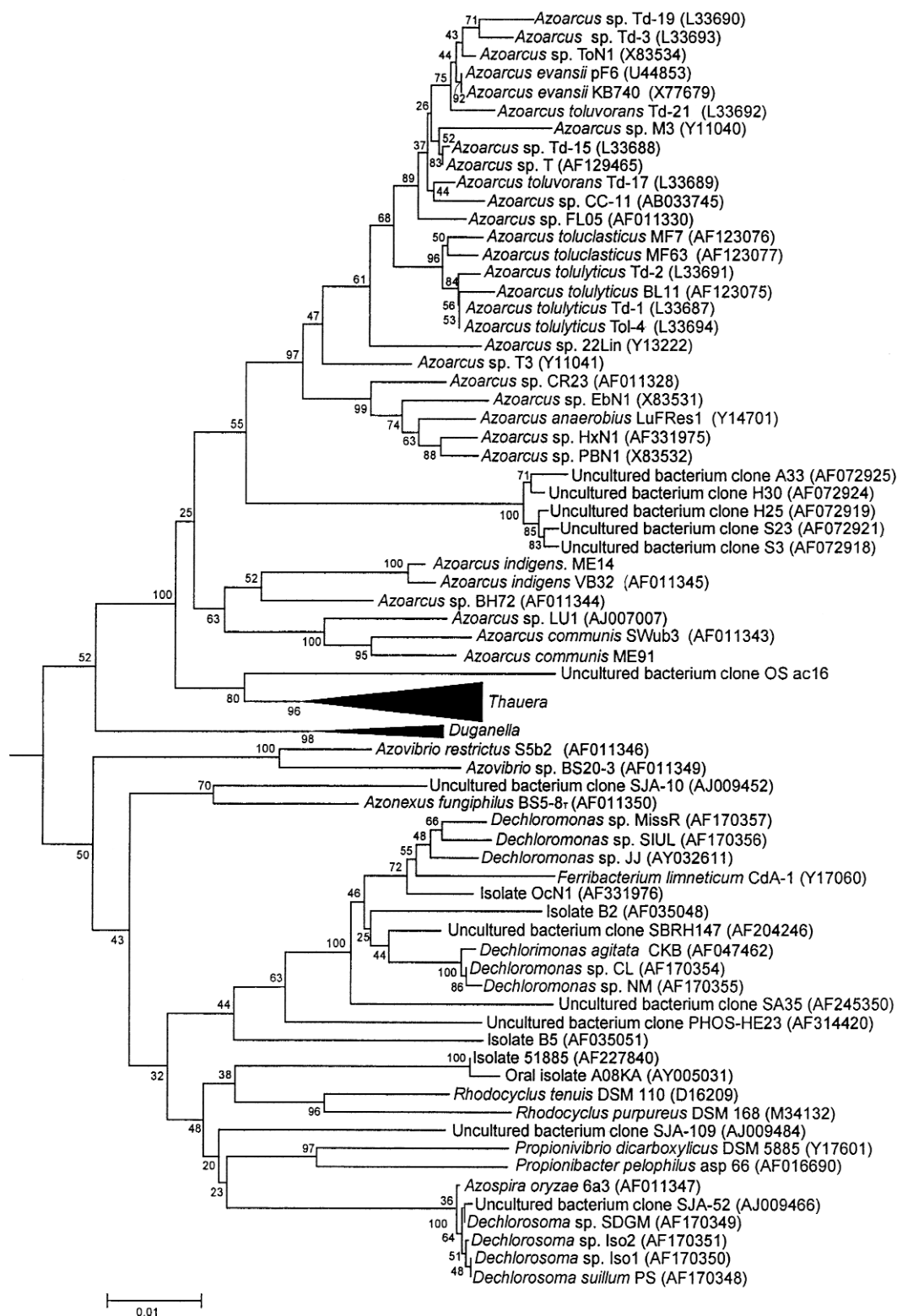
FIGURE BXII.β.106 Phylogenetic analysis of 16S rDNA sequences (1358 positions). Shown is a subtree of the *Rhodocyclus/Thauera/Azoarcus* group derived from an analysis of 158 sequences of the *Betaproteobacteria*. Tree inference was carried out using the neighbor-joining algorithm with a Jukes-Cantor correction with 125 bootstrap repetitions. Sequence accession numbers are given in parentheses.
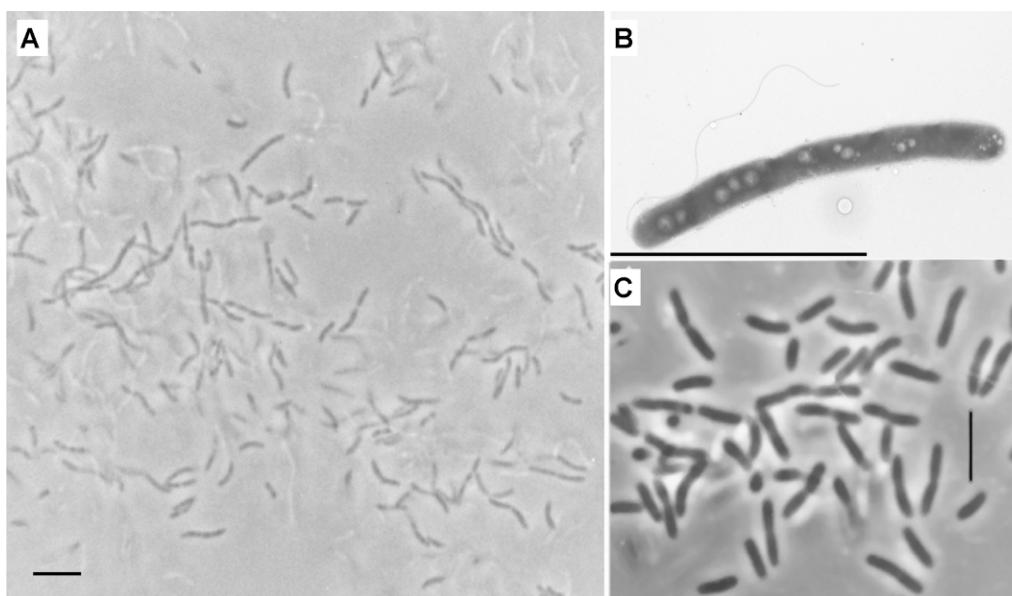
FIGURE BXII.β.107. Phase contrast (*A*, *C*) and transmission electron (*B*) microscopic images. (*A*) *Azoarcus indigens* VB32^T cultured on N$_2$ in semisolid SM medium for 24 h. (*B*) *Azoarcus* sp. strain BH72 cultured in liquid VM medium for 24 h. (*C*) *Azoarcus tolulyticus* BL2 grown on nutrient plates for 48 h. Bars, 5 μm. (Reproduced with permission from B. Reinhold-Hurek et al., International Journal of Systematic Bacteriology *43*:574–584, 1993, ©International Union of Microbiological Societies (*A*, *B*); (Reproduced with permission from B. Song, International Journal of Systematic Bacteriology *49:* 1129–1140, 1999, ©International Union of Microbiological Societies, (*C*).)

TABLE BXII.β.112. Differential characteristics of the genera *Azoarcus, Azovibrio, Azospira, Azonexus,* and morphologically similar diazotrophs or related bacteria of the *Proteobacteria*[a]

Characteristic	Azoarcus	Azonexus	Azospira	Azovibrio	Azospirillum	Burkholderia vietnamiensis	Herbaspirillum	Gluconacetobacter diazotrophicus	Thauera
Class									
Alphaproteobacteria	−	−	−	−	+	−	−	+	−
Betaproteobacteria	+	+	+	+	−	+	+	−	+
Cells curved	+	+	+	+	−	−	+	−	−
Cell width, μm	0.4–1.0[b]	0.6–0.8	0.4–0.6	0.6–0.8	0.8–1.4	0.3–0.8	0.6–0.7	0.7–0.9	0.7–1.0
Colony color	Yellow to beige	Ochreous	Pink translucent	Beige	Pink opaque	Cream	Cream	Brown	White-yellow[c]
Fermentative	−	−	−	−	D	−	−	+	−[d]
Growth on sugars	D[e]	−	−	−	+	+	+	+	−[f]
Requirement for cobalamine	−	+	−	−	−	−	−	−	−
Growth on:									
n-Butylamine	+[g]	−	−	−		−	D		
n-Caproate	D	−	+	−		+	−		D
Glutarate	+[h]	−	−	−		+	−		+[i]
4-Hydroxybenzoate	+[j]	−	−	−		+	+		+
D-Mannose	−	−	−	−	D	+	+		
Phenylacetate	+[k]	−	−	−	−	+	−		+
L-Proline	D	+	−	−	+	+	+	+	+[d]
Propionate	D	−	+	+	+	+	+		+[d]
2-Oxoglutarate	+[l]	+	+	−	D	d	+		

[a]Symbols: see standard definitions. Blank space, not determined.

[b]Except for *A. anaerobius,* whose cells are 1.5 μm.

[c]Several strains do not grow on nutrient agar.

[d]Tested for *T. aromatica.*

[e]Positive for soil-borne species, negative for plant-associated species.

[f]Except for *T. selenatis.*

[g]ND for *A. toluvorans, A. toluclasticus, A. anaerobius.*

[h]Negative for *A. indigens,* ND for *A. toluvorans, A. toluclasticus, A. anaerobius.*

[i]D for *T. mechernichensis.*

[j]Negative for *A. evansii.*

[k]Negative for *A. toluclasticus,* d for *A. tolulyticus.*

[l]Negative for *A. tolulyticus,* ND for *A. toluvorans, A. toluclasticus, A. anaerobius.*

Broad host range plasmids based on RK2 such as pRK290 or pAFR3 can be transferred by triparental mating and are stably replicated in strain BH72 (Egener et al., 2001). Transformation can also be achieved by electroporation (Hurek et al., 1995), and mutagenesis by allelic exchange (Hurek et al., 1995) or transposon mutagenesis (Dörr et al., 1998) is possible.

Antibiotic resistance Resistance of *Azoarcus* species to antibiotics has not been extensively tested. *A. indigens*, *A. communis*, and strain BH72 are not resistant to ampicillin, kanamycin, streptomycin, spectinomycin, or tetracycline (Reinhold-Hurek and Hurek, unpublished observation).

Pathogenicity None of the strains has been reported to be pathogenic. Although the plant-associated species colonize the interior of grasses as endophytes, no symptoms of plant disease have been reported (Reinhold-Hurek and Hurek, 1998).

Ecology The two groups of *Azoarcus* species—plant-associated and soil-borne species—differ strongly in their ecology. Strains of *A. indigens*, *A. communis*, and *Azoarcus* sp. BH72 occur inside roots or on the root surface of Gramineae and have never been isolated from root-free soil (Reinhold-Hurek and Hurek, 1998), except for a strain of *A. communis* that originated not from plants but from French refinery oily sludge (Laguerre et al., 1987; Reinhold-Hurek et al., 1993b). In contrast, all isolates that belong to the clade of *A. tolulyticus*, *A. toluvorans*, *A. toluclasticus*, *A. evansii*, or *A. anaerobius* have not been isolated from living plants but instead from soil and sediments; therefore, their ecology will be treated separately. The plant-associated strains were originally detected in association with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth), a flood-tolerant salt marsh grass grown as a pioneer species on salt-affected, flooded, low-fertility soils in the Punjab of Pakistan since the 1970s (Reinhold et al., 1986; Reinhold-Hurek et al., 1993b). These nitrogen-fixing *Azoarcus* strains were found in high numbers in surface-sterilized roots and only rarely on the root surface (*A. communis*). Isolates were later obtained from rice (*Oryza sativa*) from Nepal (Engelhard et al., 2000) or from resting stages (sclerotia) of a plant-associated basidiomycete found in rice field soil from Pakistan (Hurek et al., 1997b). More detailed studies on plant–microbe interactions of a strain originating from Kallar grass—strain BH72—showed that in gnotobiotic culture in the laboratory, these bacteria had a wider host range. They could also invade rice roots and stems (Hurek et al., 1994b), where they mainly colonize the cortex tissue intercellularly and, rarely, the stele including xylem cells (Hurek et al., 1994b). Despite a high density of colonization, the bacteria do not cause symptoms of plant disease and thus have an endophytic and not a pathogenic lifestyle. Unlike rhizobia, they do not form an endosymbiosis in living plant cells. Nevertheless, they show endophytic nitrogen fixation, expressing nitrogenase genes in the apoplast of aerenchymatic air spaces of flooded rice seedlings (Egener et al., 1999) or field-grown Kallar grass plants (Hurek et al., 1997a). They might even be distributed, based on molecular-ecological studies on root material or fungal spores. *Azoarcus* 16S rDNA genes (Hurek and Reinhold-Hurek, 1995) or *nifH* genes (Ueda et al., 1995; Engelhard et al., 2000) have been retrieved that did not correspond to the genes of cultivated strains or species. Because the corresponding bacteria could not be isolated from the same samples, they may occur in an as yet uncultivable state and thus be overlooked by classical microbiological techniques. Interestingly, the sequences that have been retrieved so far from plant material have not clustered with genes from soil-borne species, confirming that they do not appear to be plant-associated.

The soil-borne species are very widespread. Strains belonging to the valid species have been isolated from uncontaminated soils (Song et al., 1999), from soils containing unknown contaminants in industrial areas (Song et al., 1999), and from soils containing known contaminants such as petroleum (Fries et al., 1994; Zhou et al., 1995). Many strains have also been cultured from sediments of uncontaminated or contaminated creeks (Anders et al., 1995), aquifers (Fries et al., 1994, 1997; Zhou et al., 1995), or activated sewage sludge (Springer et al., 1998). Soil-borne *Azoarcus* spp. are also widespread with respect to their geographical distribution. For example, they have been found in North America (USA and Canada), South America (Puerto Rico, Brazil), and Europe (Germany, Switzerland). Their occurrence in anoxic sediments or sewage sludge indicates that their lifestyle *in situ* might be anaerobic (using nitrate as terminal electron acceptor) rather than microaerobic (fixing nitrogen, and using O_2 as terminal electron acceptor) in contrast to the plant-associated strains. Further information on strains that do not belong to validly published species is given below (see Other Organisms).

ENRICHMENT AND ISOLATION PROCEDURES

Plant-associated species and soil-borne species of *Azoarcus* will be treated separately. Plant-associated strains are best enriched on media free of combined nitrogen. Washed roots or roots that have been surface-sterilized in 5% NaOCl for 2 min and then thoroughly washed are macerated aseptically in the enrichment medium free of carbon source. To avoid overgrowth by faster growing diazotrophs, enrichment cultures should be inoculated with serial dilutions of this material. For enrichment of nitrogen-fixing bacteria, N-free semisolid synthetic malate medium (SM-N) is used.[2] The medium is solidified in screw-cap tubes and is inoculated with 10 µl of macerate or root pieces below the medium surface. The tubes are incubated without shaking at 30°C (37°C for tropical or subtropical strains) and checked for development of a subsurface pellicle. Samples for streaking should be taken before the pellicle has moved to the medium surface and become very dense. Isolation of single colonies is carried out on SM-N agar supplemented with 20 mg of yeast extract per liter and 10 g of agar per liter. Enrichment cultures or isolates are analyzed for nitrogenase activity in semisolid medium by the acetylene reduction method. Purity and colony color are checked on a complex medium, VM ethanol, which is based on SM-N medium but supplemented with the following ingredients (per liter): Lab Lemco Powder (Oxoid) or Bacto Peptone (Difco), 3.0 g; yeast extract, 1.0 g; NaCl, 1.0 g; NH₄Cl, 0.5 g; and agar, 15.0 g. Alternatively, for salt-affected habitats, the SSM medium of Reinhold et al. (1986) can be used instead of SM medium for enrichment and isolation.

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2. N-free semisolid synthetic malate medium (SM-N) consist of (per liter of distilled water): $MgSO_4 \cdot 2H_2O$, 0.2 g; NaCl, 0.1 g; $CaCl_2$, 0.02 g; $MnSO_4 \cdot H_2O$, 0.01 g; $Na_2MoO_4 \cdot 2H_2O$, 0.002 g; Fe(III)-EDTA, 0.066 g; phosphate buffer consisting of KH_2PO_4, 0.6 g, and K_2HPO_4, 0.4 g, which is adjusted to pH 6.8; DL-malate solution consisting of DL-malic acid, 5 g; and KOH, 4.5 g, adjusted to pH 6.8; agar, 2 g; and vitamin solution, 1 ml. The vitamin solution contains (mg per liter of distilled water) myoinositol, 10,000; niacinamide, 100; pyridoxine-HCl, 100; thiamine-HCl, 100; calcium pantothenate, 50; folic acid, 20; choline chloride, 50; riboflavin, 10; ascorbic acid, 100; *p*-aminobenzoic acid, 1.0; vitamin A, 0.5; vitamin D_3, 0.5; vitamin B_{12}, 0.5; and D-biotin, 0.5. Phosphate buffer and vitamin solution are sterilized separately and added to the autoclaved medium after autoclaving.

For soil-borne strains, enrichment is routinely done under strictly anaerobic conditions with nitrate as the terminal electron acceptor. A variety of aromatic carbon sources has been used, depending on the species or strain under study. The preparation of media and cultivation of bacteria are carried out under strictly anoxic conditions. A typical medium is that used for *A. evansii*.[3] Ascorbic acid (4 mM) can be used to reduce the medium. Potentially toxic carbon sources are added at low concentrations (toluene, 5 ppm; phenol, 1 mM; Na-benzoate, 5 mM), and cultures are spiked again with the carbon source after its degradation. Enrichment is carried out in anoxic sealed serum bottles. When rich sediments are used as an inoculum, they may have to be depleted of readily oxidizable carbon sources by repeated incubation in a medium free of these sources (Fries et al., 1994). The enriched samples are plated on agar media such as M-R2A medium[4] or used for an agar dilution series (Widdel and Bak, 1992). For cultivation on poorly water-soluble compounds such as alkylbenzenes, special procedures are required (see Rabus and Widdel, 1995).

Maintenance Procedures

Due to alkalinization of the medium in stationary phase, subculturing *Azoarcus* strains on the salts of malic acid should be avoided and VM ethanol medium should be used instead (soil-borne strains may also be subcultured on M-R2A medium).

Preservation can be done by lyophilization. Strains may also be stored in liquid nitrogen, with 5% DMSO as a cryoprotectant.

Procedures for Testing Special Characters

The genus *Azoarcus* can be specifically detected by 16S rDNA-targeted PCR. Primers TH3 (5′-GATTGGAGCGGCCGATGTC-3′) and TH5 (5′-CTGGTTCCCGAAGGCACCC-3′), which correspond to *E. coli* positions 222 to 240 and 1040 to 1022, respectively, yield a specific amplification product at an annealing temperature of 70°C for 30 cycles (Hurek et al., 1993).

Differentiation of the Genus *Azoarcus* from Other Genera

Table BXII.β.112 gives the characteristics of *Azoarcus* that differentiate it from other morphologically or physiologically similar genera. The phylogenetically closely related genus *Thauera*,

which is physiologically the most similar to the soil-borne species, is difficult to differentiate by classical tests.

Taxonomic Comments

Azoarcus spp. belong to the order *Rhodocyclales* of the class *Betaproteobacteria* according to phylogenetic analysis of almost complete 16S rRNA sequences. Within the genus *Azoarcus*, the 16S rDNA phylogenetic distances are as high as 6%; thus, the genus represents a rather heterogeneous group. Especially within the phylogenetic branch containing soil-borne strains, the 16S rDNA sequence analysis does not always resolve species well from each other (see *A. toluvorans*, Fig. BXII.β.106). *Thauera* is the most closely related genus with a 16S rDNA phylogenetic distance of 6–7%.

Several members of the genus *Azoarcus sensu lato* (Reinhold-Hurek et al., 1993b) were recently reclassified as separate genera; these are *Azovibrio*, *Azonexus*, and *Azospira*, which are closely related to the *Azoarcus/Thauera* clade (Fig. BXII.β.106) (Reinhold-Hurek and Hurek, 2000). Members of the residual genus *Azoarcus* are phylogenetically most closely related to the genus *Thauera*, with which they form the *Azoarcus/Thauera* branch, which is reasonably supported by statistical analysis (Fig. BXII.β.106). *Azoarcus* species are located in two different clades, which in part reflects their physiology and ecology.

The genus *Azoarcus* consists of the following validly described species based on polyphasic taxonomic approaches including DNA–DNA hybridization, protein profiles, fatty acid analysis, and nutritional profiles: *A. indigens* (type species), *A. communis*, *A. tolulyticus*, *A. toluclasticus*, *A. toluvorans*, *A. evansii*, and *A. anaerobius*. The unnamed strain BH72 differs from these at the species level according to DNA–DNA homology studies (≤ 25% DNA binding); however, a species name has not been given because there is only a single strain (Reinhold-Hurek et al., 1993b). Numerous additional soil-borne strains are localized in the *Azoarcus* clade according to 16S rDNA sequence analysis (examples given in Fig. BXII.β.106). Most have been isolated under conditions of denitrification with aromatic hydrocarbons as carbon sources, but the lack of nutritional/physiological data and DNA–DNA homology values do not allow a species assignment. Interestingly, a deeply branching clade of as yet uncultured bacteria is also localized within the *Azoarcus* cluster.

The analysis of phylogenetic relationships in the *Azoarcus/Thauera* 16S rDNA cluster is rendered difficult because the branching pattern between *Thauera*, the soil-borne *Azoarcus* species, and the plant-associated *Azoarcus* species is unstable when tested with different tree-building methods. This was also observed previously (Hurek et al., 1997a; Reinhold-Hurek and Hurek, 2000). Similarly, in other taxa closely related to each other, such as the rhizobia, the resolution of phylogenetic analysis is sometimes limited. Nevertheless, all named species in the genus *Thauera* cluster in one clade with a significant level of support by bootstrap analysis (Fig. BXII.β.106), allowing a reliable assignment to this genus. Likewise, most soil-borne species and strains of *Azoarcus* fall into one clade significantly supported by bootstrap analysis, and none of the species containing plant-associated strains are located on this branch. The phylogenetic distances within these three clades are similar; the consequence of this finding—that the clades might deserve the rank of different genera—is discussed below. All of the valid species of *Azoarcus* are well resolved except for the strains of *A. toluvorans*, which are interspersed among several strains of uncertain affiliation (Fig. BXII.β.106). Former strains of *A. tolulyticus*—Td-3 and Td-

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3. *Azoarcus evansii* medium consists of (g per liter of distilled water): KH$_2$PO$_4$, 0.816 g; K$_2$HPO$_4$, 5.920 g; NH$_4$Cl, 0.53 g; MgSO$_4$·7H$_2$O, 0.200 g; KNO$_3$, 0.5 g; and CaCl$_2$·2H$_2$O, 0.025 g. The phosphate is dissolved separately from the other ingredients. Both solutions are adjusted to pH 7.8 for *A. evansii* or to pH 7 for other strains. The two solutions are autoclaved and combined after cooling. To this solution are added 10 ml of sterile trace elements SL-1 and 5 ml of vitamin solution. SL-1 solution contains (per liter of distilled water): HCl (25%; 7.7 M), 10 ml; FeCl$_2$·4H$_2$O, 1.5 g; ZnCl$_2$, 70 mg; MnCl$_2$·4H$_2$O, 100 mg; H$_3$BO$_3$, 6 mg; CoCl$_2$·6H$_2$O, 190 mg; CuCl$_2$·2H$_2$O, 2 mg; NiCl$_2$·6H$_2$O, 24 mg; and Na$_2$MoO$_4$·2H$_2$O, 36 mg. The FeCl$_2$ is dissolved in the HCl and then diluted in water. The other salts are then added and dissolved. The vitamin solution contains (mg per liter of distilled water): vitamin B$_{12}$, 50 mg; pantothenic acid, 50 mg; riboflavin, 50 mg; pyridoxamine-HCl, 10 mg; biotin, 20 mg; folic acid, 20 mg; nicotinic acid, 25 mg; nicotinamide, 25 mg; α-lipoic acid, 50 mg; *p*-aminobenzoic acid, 50 mg; and thiamine-HCl·2H$_2$O, 50 mg.

4. M-R2A medium (Fries et al., 1994) contains (per liter of distilled water): yeast extract, 0.5 g; peptone, 0.5 g; Casamino acids, 0.5 g; dextrose, 0.5 g; soluble starch, 0.5 g; sodium pyruvate, 0.3 g; K$_2$HPO$_4$, 0.4 g; KH$_2$PO$_4$, 0.25 g; KNO$_3$, 0.505 g; CaCl$_2$·2H$_2$O, 0.015 g; MgCl$_2$·2H$_2$O, 0.02 g; FeSO$_4$·7H$_2$O, 0.007 g; Na$_2$SO$_4$, 0.005 g; NH$_4$Cl, 0.8 g; MnCl$_2$·4H$_2$O, 5 mg; H$_3$BO$_3$, 0.5 mg; ZnCl$_2$, 0.5 mg; CoCl$_2$·6H$_2$O, 0.5 mg; NiSO$_4$·6H$_2$O, 0.5 mg; CuCl$_2$·2H$_2$O, 0.3 mg; NaMoO$_4$·2H$_2$O, 0.01 mg; agar, 15 g. The pH is adjusted to 7.0 before autoclaving.

19—have recently been removed from this species due to low DNA–DNA hybridization values (<40%), which are also reflected in the 16S rDNA sequence analysis (Fig. BXII.β.106).

In the original description of the genus *Azoarcus*, two groups of bacteria that were distinct at the species level and located on the *Azoarcus* rRNA branch—albeit at low $T_{m(e)}$ values—were classified as *Azoarcus sensu lato* (Reinhold-Hurek et al., 1993b). Availability of additional strains and 16S rDNA sequences allowed the reassessment of the taxonomic structure of *Azoarcus sensu lato*. The unnamed groups C and D (Reinhold-Hurek et al., 1993b) were classified as the genera *Azovibrio* and *Azospira*, respectively (Reinhold-Hurek and Hurek, 2000), and *Azoarcus sensu lato* group E, which had been described later (Hurek et al., 1997b), was proposed as *Azonexus* (Reinhold-Hurek and Hurek, 2000). These bacteria are very similar in physiology and ecology to the plant-associated *Azoarcus* species.

The definition of the genus *Azoarcus* is complex. It includes strains with a variety of physiological and ecological attributes, which place them into at least two groups—the plant-associated species and the soil-borne species. In addition to their ecological differences, the species containing plant-associated strains differ from the soil-borne species in some nutritional features, e.g., the inability to use carbohydrates or, under conditions of denitrification, certain aromatic compounds, as sole carbon source. Moreover, the phylogenetic analysis of 16S rDNA sequences also points to a separation of these two groups. Since the phylogenetic distances within the three clades in the *Azoarcus/Thauera* group are similar, both subgroups of *Azoarcus* might also deserve the rank of different genera in future. However, this will require a rigid polyphasic taxonomic analysis, which subjects the strains to the same tests. As can be seen from Tables BXII.β.113 and BXII.β.114, many features have not been tested for all species or strains. For instance, nitrogen fixation and aerobic nutritional profiles are not tested in all soil-borne strains, whereas nutritional profiles under conditions of denitrification are lacking for plant-associated species. These studies should also include the numerous soil-borne isolates that have not yet been assigned to any species. Because some strains are deeply branching in the phylogenetic analysis (e.g., strains 22Lin, T3, and M3; Fig. BXII.β.106) the description of new species might be expected.

FURTHER READING

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DIFFERENTIATION OF THE SPECIES OF THE GENUS *AZOARCUS*

The differential characteristics of the species of *Azoarcus* are indicated in Table BXII.β.113. Other characteristics of the species are presented in Table BXII.β.114.

List of species of the genus Azoarcus

1. **Azoarcus indigens** Reinhold-Hurek, Hurek, Gillis, Hoste, Vancanneyt, Kersters and De Ley 1993b, 583[VP]

   in′di.gens. L. v. *indigere* to be in need of; M.L. pres. part. *indigens* being in need of, referring to the vitamin requirements.

   This species can be differentiated from the other species by its requirement for *p*-aminobenzoic acid, by its ability to grow on itaconate, and by a combination of characteristics given in Table BXII.β.113. Additional characteristics are given in Table BXII.β.114. Cells are thin (0.5–0.7 μm wide) and curved; cell pairs appear slightly S-shaped. Colonies are very compact and difficult to disperse. Growth in liquid media is clumpy; aggregation is very strong on peptone media. Optimal temperature, 40°C. Diazotrophic. The major fatty acids are $C_{10:0\ 3OH}$, $C_{12:0}$, $C_{16:1\ \omega7c}$, $C_{17:0\ cyclo}$, and $C_{18:1}$. Isolated from roots and stem bases of *Leptochloa fusca* (L.) Kunth from Punjab of Pakistan (Reinhold-Hurek et al., 1993b), black sclerotia of an *Ustilago*-related basidiomycete from rice soil in the Punjab of Pakistan (Hurek et al., 1997b), and from rice roots (*Oryza sativa*) from Nepal (Engelhard et al., 2000).

   The mol% G + C of the DNA is: 62.4 (T_m).
   Type strain: VB32, DSM 12121, LMG 9092.
   GenBank accession number (16S rRNA): AF011345.

2. **Azoarcus anaerobius** Springer, Ludwig, Philipp and Schink 1998, 954[VP]

   an.a.e.ro′bi.us. Gr. pref. *an* not; Gr. n. *aer* air; Gr. n. *bios* life; N.L. adj. *anaerobius* not living in air, anaerobic.

   This species can be differentiated from the others by its strictly anaerobic lifestyle with nitrate as the only electron acceptor. Nitrate is quantitatively reduced to N_2 gas, nitrite is accumulated as an intermediate, and N_2O is not detected. Oxygen is not reduced, even at low partial pressures. Sulfate, thiosulfate, sulfite, sulfur, trimethylamine *N*-oxide, DMSO, $Fe(OH)_3$, $K_3[Fe(CN)_6]$, and fumarate are not reduced. Superoxide dismutase-positive. Additional characteristics are given in Tables BXII.β.113 and BXII.β.114. Optimal temperature, 28°C. pH range, 6.5–8.2; optimal pH, 7.2. Enhanced salt concentration impairs growth. Not diazotrophic. Sole carbon sources for growth include propanol, valerate, pyruvate, cyclohexanecarboxylate, phenol, resorcinol, and *p*-cresol. No growth occurs with L-malate, formate, 5-oxocaproate, pimelate, catechol, hydroquinone, 2-hydroxybenzoate, *o*-cresol, and *m*-cresol. No autotrophic growth occurs with hydrogen or thiosulfate. Isolated from sewage sludge.

   The mol% G + C of the DNA is: 65.5 ± 0.5 (T_m).
   Type strain: LuFRes1, DSM 12081.
   GenBank accession number (16S rRNA): Yl4701.

3. **Azoarcus communis** Reinhold-Hurek, Hurek, Gillis, Hoste, Vancanneyt, Kersters and De Ley 1993b, 583[VP]

   com′mu.nis. L. masc. adj. *communis* common, referring to diverse habitats.

TABLE BXII.β.113. Characteristics differentiating species of the genus *Azoarcus*[a]

Characteristic	A. indigens	A. anaerobius	A. communis	A. evansii	A. toluclasticus	A. tolulyticus	A. toluvorans	Unnamed strain BH72
Cell width (μm)	0.5–0.7	1.5	0.8–1.0	0.4–0.8	0.6–0.8	0.8–1.0	0.8–1.0	0.6–0.8
O₂ terminal electron acceptor	+	−	+	+	+	+	+	+
Catalase	+	−	+	+	−	d	+	+
Nitrogen fixation	+	−	−	−	−	+	−	+
Requirement for *p*-aminobenzoic acid	+	−	−	−	−	−	−	−
Sole carbon sources:								
Adipate, D-ribose	−		−	−	+	+	+	−
p-Aminobenzoate	+		−	+	−	+		−
L-Arabinose, D-xylose	−	−	−	−	+	+	+	−
n-Caproate	−		+	−	−	d	−	+
Citrate	−		+	−		−		−
D-Fructose	−	−	−	+	d	+	−	−
D-Galactose, L-proline, sucrose	−		−	+	+	+	+	−
D-Glucose	−	−	−	d	d	+	−	−
3-Hydroxybenzoate	+	+	+	+	−	d	−	+
Isovalerate	−	+	+	+	−	+		+
Itaconate	+		−	−		−	−	−
Maltose	−		−	d	+	+	+	−
D-Mandelate	+		+	−		−		−
Phenylacetate	+	+	+	d	−	d	+	+
L-Phenylalanine	+	+	+	+	d	d	±	−
D-Tartrate	+		D	+	−	−	−	+

[a]Symbols: see standard definitions. Blank space, not determined. ±, weakly positive.

This species can be differentiated from the others by its cell width (0.8–1.0 μm), by its growth on D-mandelate and on citrate, and by a combination of characteristics given in Table BXII.β.113. Additional characteristics are given in Table BXII.β.114. Cells are plump and only slightly curved. Optimal temperature, 37°C. Diazotrophic. Some strains grow well at 2% NaCl. The major fatty acids are $C_{10:0\ 3OH}$, $C_{14:0}$, $C_{16:1\ \omega7c}$, $C_{16:0}$, and $C_{18:1}$. Isolated from roots of *L. fusca* (L.) Kunth from Punjab of Pakistan (SWub3) (Reinhold-Hurek et al., 1993b; Engelhard et al., 2000) and from refinery oily sludge in France (Laguerre et al., 1987).

The mol% G + C of the DNA is: 62.4 (T_m).

Type strain: SWub3.

GenBank accession number (16S rRNA): AF011343.

Additional Remarks: The species affiliation of strain LU1, which was isolated from a compost biofilter in Canada (Juteau et al., 1999), is not clear; it has a phylogenetic distance of 2.9% with the type strain.

4. **Azoarcus evansii** Anders, Kaetzke, Kämpfer, Ludwig and Fuchs 1995, 331[VP]

e'van.si.i. L. gen. n. *evansii* of Evans, in honor of the late W.C. Evans, a pioneer in studies of anaerobic aromatic metabolism.

This species can be differentiated from other species by a combination of characters given in Table BXII.β.113. Cells are rods with rounded ends, motile by means of a subpolar flagellum. Yeast extract (0.1%) inhibits growth. Optimal temperature 35–37°C. Optimal pH, 7.8. Does not fix nitrogen. During denitrifying growth on aromatic compounds, nitrite is an intermediate and is reduced mainly to N_2O (strain KB740). Characteristics in addition to those listed in Table BXII.β.114 are as follows. Under anaerobic conditions, the type strain KB740 uses benzoate, phenylacetate, phenylglyoxylate, 3-and 4-hydroxybenzoate, 2-aminobenzoate, 4-hydroxyphenylacetate, phenylalanine, *p*-cresol, 2-fluorobenzoate, benzaldehyde, benzyl alcohol, indolylacetate, *o*-phthalate, adipate, pimelate, cyclohexanecarboxylate, succinate, fumarate, L-malate, acetate, acetone, D-fructose, and D-maltose. Slow growth occurs on glutarate and D-glucose but not on toluene, phenol, 2-hydroxybenzoate, protocatechuate, *o*-and *m*-cresol, indole, ethanol, D-ribose, and D-lactose. Pyridine is used by reference strain pF6 under aerobic and anaerobic conditions. The aerobic metabolism of benzoate is unusual in the type strain KB740, since none of the known pathways—i.e., the conversion of benzoate to catechol (1,2-dihydroxybenzoene) or protocatechuate (3,4-dihydroxybenzoate)—appear to operate in this species (Mohamed et al., 2001). The first step is the activation of benzoate to benzoyl-CoA by a benzoate-CoA ligase, and the second step involves the hydroxylation of benzoyl-CoA by a novel benzoyl-CoA oxygenase (Mohamed et al., 2001). The first step of phenylacetate degradation is catalyzed by two different phenylacetate-CoA-ligases under aerobic and anaerobic conditions, respectively (Mohamed, 2000). Reference strain PF6 degrades pyridine aerobically and anaerobically when growing on nitrate (Rhee et al., 1997). Benzoyl-CoA is also a central intermediate in the anaerobic degradation of aromatic compounds (Harwood et al., 1998). In the type strain KB740, the nucleotide sequence analysis of the gene cluster—including a gene for benzoate-CoA ligase—indicates that the degradation of benzoate is probably similar to the benzoate-CoA pathway in *Thauera aromatica* (Harwood et al., 1998). The major cellular fatty acids are $C_{16:1\ \omega7c}$, $C_{16:0}$, $C_{12:0}$, $C_{10:0\ 3OH}$, and $C_{18:1\ \omega7,\ 9c}$. The major quinone is ubiquinone 8. The species was described with one strain isolated from creek sediment in the United States (Braun and Gibson, 1984). However, a second strain,

TABLE BXII.β.114. Further characteristics of species of the genus *Azoarcus*[a]

Characteristic	*Azoarcus indigens*	*Azoarcus anaerobius*	*Azoarcus communis*	*Azoarcus evansii*[b]	*Azoarcus toluclasticus*	*Azoarcus tolulyticus*[b]	*Azoarcus toluvorans*	*Azoarcus* sp. BH72	*Azonexus fungiphilus*	*Azospira oryzae*	*Azovibrio restrictus*	*Azovibrio* sp. BS20-3
Number of strains examined	5	1	3	2	5	9	2	1	5	12	5	1
Cell width (μm)	0.5–0.7	1.5	0.8–1.0	0.4–0.8	0.6–0.8	0.8–1[b]	0.8–1.0	0.6–0.8	0.6–0.8	0.4–0.6	0.6–0.8	0.6–0.8
Cell length (μm)	2.0–4.0	2.7–3.3	1.5–3.0	1.5–3.0	1.7–4.0	1.4–2.8	1.4–2.8	1.5–4.0	1.5–4.0	1.1–2.5	1.5–3.5	1.5–3.5
Elongated cells in stationary cultures	r	ND	R	−	ND	−	ND	r	+	r	R	ND
Colony diameter (mm, VM/CR)[c]	2–3	0	2–4	0.2–0.7[b]	ND	1–1.5[b]	ND	2–3/1	1–2/0.7	1.0–2.0	1.5–2/1	Negligible
Colony color (VM)[c]	Opaque yellowish	−	Translucent yellowish	Translucent beige[b]	ND	Opaque yellowish[b]	ND	Translucent yellowish	Opaque ocher	Translucent pink-salmon	Opaque beige	Negligible
Colony color (CR)[c]	Whitish pink; white margin	−	Whitish pink; pink center	Orange red[b]	ND	Orange red[b]	ND	Orange red	Dark red	Translucent orange	Orange red	Negligible
Colony surface	Rough	−	Smooth	Shining	ND	Shining	ND	Shining	Shining	Shining	Shining	ND
Growth at 40°C	+	−	+	−[b]	−	−[b]	−	+	+	+	d	−
Requirement for *p*-aminobenzoic acid	+	−	+	−	−	−	−	−	+	+	−	+
Requirement for cobalamin	−	−	D	−	−	−	−	−	+	d	−	+
Nitrogen fixation	+	−	+	−	+	+	ND	+	+	+	+	+
Oxidation/fermentation of glucose	−	−	−	+[d]	ND	ND	ND	−	−	−	−	−
Catalase	+	−	+	+	−	D	+	+	+	+	+	ND
Sole carbon sources for growth:[e]												
L(+) Arabinose, D(+)-xylose	−	−	−	−	+	+	+	−	−	−	−	−
Adipate	−	ND	−	−	ND	+	+	−	−	−	−	−
D-Alanine, glycerol	−	ND	D	−[b]	ND	−[b]	ND	+	−	−	−	+
L-Alanine	d	ND	+	+	ND	+[b]	ND	+	−	−	d	−
p-Aminobenzoate	+	ND	−	+	+	−[b]	ND	−	−	−	−	−
L-Arginine	−	ND	V	−	+	−	+	−	−	−	+	+
L-Aspartate	+	ND	D	+	+	+[b]	+	+	+	+	−	−
Benzoate	d	+	V	+[b]	ND	+[b]	ND	+	−	−	−	−
Benzylamine	d	ND	−	+[b]	ND	+[b]	ND	+	−	−	−	−
Betain, *meso*-tartrate[b]	−	ND	+	+[b]	ND	−	ND	−	−	−	−	−
n-Butylamine	+	ND	+	−[b]	ND	+[b]	ND	+	−	−	−	−
n-Caproate	−	ND	+	−	−	−[b]	−	+	−	+	−	−
Citrate	−	ND	+	−[b]	ND	d	ND	−	−	−	−	−
m-Coumarate	d	ND	+	−	ND	−[b]	ND	+	−	+	−	−
D(+)-Fructose	−	−	−	+[b]	d	+	−	−	−	−	−	−

(continued)

TABLE BXII.β.114. *(cont.)*

Characteristic	*Azoarcus indigens*	*Azoarcus anaerobius*	*Azoarcus communis*	*Azoarcus evansii* [b]	*Azoarcus toluclasticus*	*Azoarcus tolulyticus* [b]	*Azoarcus toluvorans*	*Azoarcus* sp. BH72	*Azonexus fungiphilus*	*Azospira oryzae*	*Azovibrio restrictus*	*Azovibrio* sp. BS20-3
Fumarate	+	+	+	+	d	+	+	+	+	+	+	+
D(+)-Galactose, sucrose	–	ND	–	+[b]	+	+[b]	+	–	+	+	+	–
Gentisate	+	ND	+	+	ND	–[b]	ND	+	–	–	–	–
D(+)-Glucose	–	–	–	d	d	+	–	–	–	–	v	+
Glutarate	d	ND	+	+[b]	ND	+[b]	ND	+	–	–	–	–
DL-Glycerate	+	ND	–	+[b]	ND	+[b]	ND	–	–	–	–	–
3-Hydroxybenzoate	+	+[f]	+	+[b]	–	d	–	+	–	–	–	+
4-Hydroxybenzoate	+	+[f]	+	–	+[f]	+[f]	+[f]	+	–	+	–	+
3-Hydroxybutyrate	+	ND	+	+[b]	ND	+[b]	ND	+	+	v	d	+
Isobutyrate	–	+	+	+[b]	–	+[b]	±	+	–	+	–	+
Isovalerate	+	+	–	–[b]	ND	–[b]	ND	+	–	+	–	–
Itaconate	–	ND	+	–	+	+	d	–	–	–	–	–
Lactose	+	ND	+	–[b]	ND	–[b]	ND	+	–	–	–	+
L-Mandelate, protocatechuate,	+	ND	+	–	ND	+[b]	ND	+	–	–	–	–
D(+) Malate	+	–	+	–[b]	ND	+[b]	ND	+	–	–	d	–
L-Malate	+	ND	+	+	ND	+	ND	+	+	+	+	+
Malonate	–	ND	–	–	ND	–	ND	–	d	–	–	–
Maltose	–	ND	–	d	+	+	+	–	–	–	–	–
Maltotriose, palatinose, D(+)-melezitose	–	ND	–	+[b]	ND	+	ND	–	–	–	–	–
D-Mandelate	+	ND	+	–[b]	ND	–[b]	ND	–	–	–	–	–
2-Oxoglutarate	+	ND	+	+[b]	ND	–[b]	ND	+	+	+	–	–
Phenylacetate	+	+	+	d	–	d	+	+	–	–	–	–
L-Phenylalanine	+	ND	+	+	d	d	±	–	+	–	–	–
L-Proline	–	+	+	+	+	d	+	–	–	–	–	–
Propionate	+	+	+	–	–	d	–	+	+	+	+	+
(-)-Quinate	–	ND	D	–	ND	–[b]	ND	–	–	–	–	–
D(-)-Ribose	–	ND	–	–[b]	+	d	+	–	–	v	–	–
D-Tartrate	+	ND	D	+[b]	–	+	–	+	–	+	–	–
L(+)-Tartrate	–	ND	–	–[b]	–	+	+	–	–	–	–	–
Toluene (denitrifying)	–	ND	–	ND	d	–	+	+	–	–	–	–
Tryptamine	+	ND	V	–[b]	ND	+	ND	+	–	–	–	–
L-Tyrosine	+	+	+	–	–	–[b]	–	–	–	–	–	–

[a] Data from Reinhold-Hurek et al. (1993b); Anders et al. (1995); Hurek and Reinhold-Hurek (1995); Zhou et al. (1995); Hurek et al. (1997a); Rhee et al. (1997); Springer et al. (1998); Song et al. (1998); Engelhard et al. (2000); Reinhold-Hurek and Hurek (2000). For all characteristics: ±, intermediate reaction; r, rare; ND, not determined. All strains have the following features: cells are straight to curved rods; oxidase positive; no growth in the presence of 5% NaCl and no growth-rate increase when NaCl is added to medium (ND for *A. toluvorans*, *A. anaerobius*); denitrification (ND for *Azonexus* sp.); no spore formation; no starch hydrolysis (ND for *A. evansii* and *A. anaerobius*).

[b] Marked characters were determined for *A. tolulyticus* strain Td-1 or *A. evansii* strain KB740 (Reinhold-Hurek and Hurek, 2000).

[c] Growth on VM ethanol agar (VM) or Congo red agar (CR) at 37°C for 4 d.

[d] Not tested for strain pF6

[e] All strains grow on DL-lactate, succinate, acetate, L-glutamate, butyrate, ethanol (ND for *A. toluvorans* and *A. tolulyticus*) (no substrate but L-malate tested for *A. evansii* pF6); no growth on D(+)-mannose, maltitol, N-acetyl-D-glucosamine, D-gluconate, caprate; no growth on[b] (ND for *A. toluclasticus*, *A. toluvorans*, *A. anaerobius*) D(+)-trehalose, α-D(+)-melibiose, D(+)-sorbose, α-D(+)-melibiose, lactulose, 1-O-methyl-β-galactopyranoside, 1-O-methyl-α-galactopyranoside, D(+)-cellobiose, β-gentiobiose, 1-O-methyl-β-D-glucopyranoside, α-L-rhamnose, α-L-fucose, D(+)-arabitol, xylitol, dulcitol, D-tagatose, myo-inositol, D-sorbitol, adonitol, hydroxyquinoline-β-glucuronide, D-lyxose, erythritol, 1-O-methyl-α-D-glucopyranosid, 3-O-methyl-glucopyranose, D-saccharate, mucate, D-galacturonate, D-glucuronate, 5-keto-D-gluconate, 2-keto-D-gluconate, 3-phenylpropionate, caprylate, 4-aminobutyrate, DL-α-amino-n-valerate, trigonelline, putrescine, ethanolamine, D-glucosamine.

[f] Under denitrifying conditions.

pF6, sharing 100% 16S rDNA sequence identity with the type strain, was isolated on pyridine from industrial wastewater in Korea (Rhee et al., 1997).

The mol% G + C of the DNA is: 67.5.

Type strain: DSM 6898, KB740.

GenBank accession number (16S rRNA): X77679.

5. **Azoarcus toluclasticus** Song, Häggblom, Zhou, Tiedje and Palleroni 1999, 1139[VP]

to.lu.clas′ ti.cus. N.L. n. Fr. Sp. *tolu* balsam from Santiago de Tolu, toluene; Gr. adj. *clasticus* breaking; M.L. adj. *toluclasticus* toluene-breaking.

This species can be differentiated from the other species by the lack of catalase activity despite its aerobic growth, and also by a combination of characters given in Table BXII.β.113. Additional characteristics are given in Table BXII.β.114. Cells are short motile rods. Optimal temperature, 30°C. Under denitrifying conditions, growth occurs on acetate, benzoate, pyruvate, succinate, D-xylose, L-arabinose, D-ribose, D-galactose, sucrose, lactose, maltose, adipate, lactate, mannitol, aspartate, proline, and arginine. All strains except strain MF23 can use toluene as a growth substrate; strains MF58 and MF63T can also grow on phenol under denitrifying conditions. The strains grow on brain-heart infusion, nutrient and trypticase soy agar, except for strain MF63T, which does not grow on nutrient agar. The predominant fatty acids are $C_{16:0}$ and $C_{16:1 \, \omega 7c}$. DNA–DNA hybridizations show intermediate similarities (47–55%) among genomovar I (strains MF7 and MF23) and genomovar II (strains MF58, MF63T, and MF441). Isolated from aquifer sediments in the United States.

The mol% G + C of the DNA is: 67.3 (HPLC).

Type strain: MF63, ATCC 700605.

GenBank accession number (16S rRNA): AF123077.

6. **Azoarcus tolulyticus** Zhou, Fries, Chee-Sanford and Tiedje 1995, 505[VP]

to.lu.ly′ ti.cus. N.L. n. Fr. Sp. *tolu* balsam from Santiago de Tolu, toluene; Gr. adj. *lyticus* dissolving; N.L. masc. adj. *tolulyticus* toluene dissolving.

This species can be differentiated from the other species by a combination of characters given in Table BXII.β.113. Additional characteristics are given in Table BXII.β.114. Cells are short motile rods that are slightly elongated (to 2.8 μm) when grown on M-R2A agar. Diazotrophic. Optimal temperature, 30°C; growth can occur at 37°C. Under denitrifying conditions, growth occurs on acetate, adipate, arginine, L-arabinose, aspartate, benzoate, fumarate, D-galactose, D-glucose, lactate, lactose, maltose, mannitol, proline, pyruvate, D-ribose, succinate, sucrose, toluene, D-xylose, and 4-hydroxybenzoate. Growth occurs on brain-heart infusion agar but either not at all or only poorly on nutrient and trypticase soy agar. The predominant fatty acids are $C_{16:0}$ and $C_{16:1 \, \omega 7c}$. Isolated from aquifer sediments and petroleum-contaminated soils in the United States (Fries et al., 1994, 1997; Chee-Sanford et al., 1996).

The mol% G + C of the DNA is: 66.9 (HPLC).

Type strain: Tol-4, CC 51758.

GenBank accession number (16S rRNA): L33694.

Additional Remarks: Strains Td-19 and Td-3 (Zhou et al., 1995) have been removed from the species due to low DNA–DNA hybridization values with the type strain (Song

et al., 1999). Other strains of *A. tolulyticus* include strains MF66, 2a1, 3a1, 7a1, BL2, and BL11. The cells of strain MF66 are short motile rods with monopolar flagellation. This strain can grow under aerobic and denitrifying conditions with acetate, benzoate, butyrate, D-fructose, fumarate, D-glucose, phenol, pyruvate, succinate, toluene, D-xylose, L-arabinose, D-ribose, D-galactose, sucrose, lactose, maltose, adipate, lactate, mannitol, aspartate, proline, phenylalanine, or arginine. Under denitrifying conditions, 4-hydroxybenzoate is used as a growth substrate. The cells are oxidase-positive and catalase-negative and grow on brain-heart infusion plates, but do not grow on nutrient and trypticase soy agar plates. The predominant fatty acids are $C_{16:0}$ and $C_{16:1 \, \omega 7c}$, similar to those of other members of *A. tolulyticus*. It belongs to the species *A. tolulyticus* based on 16S rRNA gene sequence analysis, DNA–DNA hybridization, similar patterns of whole-cell proteins, and genomic DNA analysis. Strains 2a1, 3a1, 7a1, BL2, and BL11 were isolated from northern Michigan, USA, and are short motile rods with monopolar flagellation. They can grow on acetate, benzoate, butyrate, D-fructose, fumarate, D-glucose, pyruvate, succinate, D-xylose, L-arabinose, D-ribose, D-galactose, sucrose, lactose, maltose, adipate, lactate, mannitol, aspartate, proline, or arginine under aerobic and denitrifying conditions and use 4-hydroxy-benzoate and toluene for growth under denitrifying conditions. They grow on brain-heart infusion, nutrient, and trypticase soy agar plates, and give positive reactions in the catalase and oxidase tests. A different colony morphology of these strains on half-strength trypticase soy agar plus nitrate at 30°C for 48 h has been reported (Chee-Sanford et al., 1996). The predominant fatty acids are $C_{16:0}$ and $C_{16:1 \, \omega 7c}$. Whole cell protein and genomic DNA fragmentation analyses show identical patterns to the other members of *A. tolulyticus*, and 16S rRNA sequence analysis of one strain (BL11) shows a close relationship with the members of *A. tolulyticus*. Thus, they belong to the species *A. tolulyticus*. Because the DNA hybridization values between these isolates and the strains of *A. tolulyticus* are approximately 55%, they constitute a genomovar of this species.

7. **Azoarcus toluvorans** Song, Häggblom, Zhou, Tiedje and Palleroni 1999, 1139[VP]

to.lu.vo′ rans. N.L. n. Fr. Sp. *tolu* balsam from Santiago de Tolu, toluene; L. part. adj. *vorans* devouring; M.L. part. adj. *toluvorans* toluene-devouring.

This species can be differentiated from the other species by a combination of characters given in Table BXII.β.113. Additional characteristics are given in Table BXII.β.114. Optimal temperature, 30°C. Growth occurs on brain-heart infusion, nutrient, and trypticase soy agar. Under denitrifying conditions, growth occurs on acetate, benzoate, butyrate, fumarate, phenylacetate, pyruvate, succinate, toluene, D-xylose, L-arabinose, D-ribose, D-galactose, sucrose, maltose, adipate, lactate, mannitol, aspartate, proline, phenylalanine, arginine, 4-hydroxybenzoate, and phenol, and under aerobic conditions on benzene or ethylbenzene. Isolated from soil from industrial area in Brazil (Td17) and uncontaminated organic soil in the United States (Td21) (Fries et al., 1994).

The mol% G + C of the DNA is: 67.8 (HPLC).

Type strain: Td21, ATCC 700604.

GenBank accession number (16S rRNA): L33692.

Other Organisms

1. *Azoarcus* sp. strain BH72 Reinhold-Hurek, Hurek, Gillis, Hoste, Vancanneyt, Kersters and De Ley 1993b, 582.

This unnamed strain differs at the species level by having a DNA–DNA hybridization value of ≤25% with other species. It can be differentiated from the other species by its lack of growth on L-phenylalanine and a combination of characteristics given in Table BXII.β.113. Additional characteristics are given in Table BXII.β.114. Cells are long, thin (0.6–0.8 µm wide) and slightly curved, elongated (8–12 µm) cells occurring in late log or in stationary phase culture on N-free or ammonium-supplemented SM medium. The optimal temperature for growth is 40°C. Diazotrophic. The major fatty acids are $C_{16:0}$, $C_{16:1 \, \omega 7c}$, $C_{18:1}$, $C_{18:1}$, and $C_{14:0}$. Isolated from the root interior of *Leptochloa fusca* (L.) Kunth from Punjab of Pakistan (Reinhold et al., 1986).

The mol% G + C of the DNA is: 67.6 (T_m).

GenBank accession number (16S rRNA): AF011344.

Additional Remarks: A wide range of strains have been isolated under conditions of denitrification, mostly on aromatic carbon sources, which cluster within the clade of soil-borne *Azoarcus* species according to phylogenetic analysis of 16S rDNA sequences (Fig. BXII.β.106); however, data are not sufficient for the assignment to given species. As described above, some are only distantly related and may thus deserve the rank of separate species in future. Examples of strains and habitats are: EbN1, PbN1, isolated on ethylbenzene from freshwater mud, Germany (Rabus and Widdel, 1995); Lin22, isolated on cyclohexane-1,2-diol from activated sludge, Germany (Harder, 1997); pCyN1, isolated on *p*-cymene from freshwater mud, Germany (Harms et al., 1999a); CC-11, isolated from a 3-year-enrichment culture on phenol, Japan (Shinoda et al., 2000); M3, isolated on toluene from a diesel-fuel contaminated aquifer, Switzerland (Hess et al., 1997); CR23, isolated on phenol from creek in Costa Rica (van Schie and Young, 1998).

Genus III. **Azonexus** *Reinhold-Hurek and Hurek 2000, 658*[VP]

THE EDITORIAL BOARD

A.zo' nex.us. Fr. n. *azote* nitrogen; L. masc. n. *nexus* coil; M.L. masc. n. *Azonexus* nitrogen-fixing coil.

Gram-negative somewhat curved motile rods (0.6–0.8 × 1.5–4.0 µm). **Cells elongated and coiled in stationary phase.** One polar flagellum. Chemoorganoheterotrophic and aerobic; microaerophilic when fixing nitrogen. Strictly respiratory; O_2 is the electron acceptor. Oxidase and catalase positive. **Fix N_2.** Require cobalamine. Grow on acetate, ethanol, fumarate, DL-lactate, L-malate, 2-oxoglutarate, L-proline, and succinate.

The mol% G + C of the DNA is: not known.

Type species: **Azonexus fungiphilus** Reinhold-Hurek and Hurek 2000, 658.

FURTHER DESCRIPTIVE INFORMATION

Azonexus fungiphilus is notable for its tendency to produce elongated (up to 50 µm) cells in early stationary phase cultures (Reinhold-Hurek and Hurek, 2000). These elongated cells appear to break up into curved segments later in stationary phase.

Azonexus fungiphilus strains were recovered from fungal resting stages obtained from the rhizosphere of rice plants in Pakistan (Hurek et al., 1997b).

Analysis of 16S rDNA sequences placed *Azonexus* in the *Betaproteobacteria*; in these analyses, *Azonexus* was most closely related to members of the genera *Thauera* and *Rhodocyclus*. *Azonexus* strains could be separated from *Azoarcus*, *Azospira*, and *Azovibrio* strains by comparisons of fatty acid composition, SDS-soluble protein electrophoretic patterns, and BOX-PCR genomic fingerprinting. DNA–DNA hybridization results supported these divisions (Hurek et al., 1997b; Reinhold-Hurek and Hurek, 2000).

ENRICHMENT AND ISOLATION PROCEDURES

Enrichment and isolation procedures are described in Reinhold et al. (1986).

MAINTENANCE PROCEDURES

Maintenance and long-term storage procedures are described in Reinhold-Hurek et al. (1993b).

DIFFERENTIATION OF THE GENUS *AZONEXUS* FROM OTHER GENERA

Reinhold-Hurek and Hurek (2000) provide tables of characteristics that differentiate the genera *Azoarcus*, *Azonexus*, *Azospira*, and *Azovibrio* from each other and from *Acetobacter diazotrophicus*, *Burkholderia vietnamensis*, the genus *Azospirillum*, and the genus *Herbaspirillum* (see also Table BXII.β.112 in the chapter on *Azoarcus*).

TAXONOMIC COMMENTS

This genus was first described as *Azoarcus* sp. group E (Hurek et al., 1997b; Reinhold-Hurek and Hurek, 2000).

List of species of the genus Azonexus

1. **Azonexus fungiphilus** Reinhold-Hurek and Hurek 2000, 658[VP]

fun.gi' phil.us. M.L. masc. n. *fungi* mushrooms, fungi; Gr. adj. *philos* loving; M.L. masc. adj. *fungiphilus* loves mushrooms or fungi, referring to its source of isolation.

Description as for the genus with the following additional characteristic: grows on L-aspartate, L-glutamate, and 3-hydroxybutyrate.

The mol% G + C of the DNA is: unknown.

Type strain: BS5-8.

GenBank accession number (16S rRNA): AF011350.

Genus IV. **Azospira** Reinhold-Hurek and Hurek 2000, 658[VP]

A.zo′ spi.ra. Fr. n. *azote* nitrogen; L. fem. n. *spira* winding, turn; M.L. fem. n. *Azospira* nitrogen-fixing spiral.

Gram-negative curved motile rods (0.4–0.6 × 1.1–2.5 µm). One polar flagellum. **Cells elongated in stationary cultures.** Chemoorganoheterotrophic and aerobic; microaerophilic when fixing N_2. Strictly respiratory; O_2 and nitrate are electron acceptors. Oxidase and catalase positive. **Fix N_2.** Grow on acetate, *n*-caproate, ethanol, fumarate, L-glutamate, DL-lactate, L-malate, 2-oxoglutarate, propionate, and succinate.

The mol% G + C of the DNA is: 65–66.

Type species: **Azospira oryzae** Reinhold-Hurek and Hurek 2000, 658.

FURTHER DESCRIPTIVE INFORMATION

Azospira oryzae cells become elongated (up to 8 µm) in stationary phase cultures and acquire at least one helical turn (Reinhold-Hurek and Hurek, 2000).

Azospira oryzae 6a3 was isolated from the roots of Kallar grass collected in Pakistan (Reinhold-Hurek et al., 1993b). Other strains have been isolated from roots of *Oryza officinalis, O. minuta,* and *O. sativa.*

Analysis of 16S rDNA sequences placed *Azospira* in the *Betaproteobacteria*; in these analyses, *Azospira* was most closely related to members of the genera *Thauera* and *Rhodocyclus*. *Azospira* strains could be separated from *Azoarcus, Azonexus,* and *Azovibrio* strains by comparisons of fatty acid composition, SDS-soluble protein electrophoretic patterns, and BOX-PCR genomic fingerprinting. DNA–DNA hybridization results supported these divisions (Hurek et al., 1997b; Reinhold-Hurek and Hurek, 2000).

ENRICHMENT AND ISOLATION PROCEDURES

Enrichment and isolation procedures are described in Reinhold et al. (1986).

MAINTENANCE PROCEDURES

Maintenance and long-term storage procedures are described in Reinhold-Hurek et al. (1993b).

DIFFERENTIATION OF THE GENUS *AZOSPIRA* FROM OTHER GENERA

Reinhold-Hurek and Hurek (2000) provide tables of characteristics that differentiate the genera *Azoarcus, Azonexus, Azospira,* and *Azovibrio* from each other and from *Acetobacter diazotrophicus, Burkholderia vietnamensis,* the genus *Azospirillum,* and the genus *Herbaspirillum* (see also Table BXII.β.112 in the chapter on *Azoarcus*).

TAXONOMIC COMMENTS

This genus was first described as *Azoarcus* sp. group D (Reinhold-Hurek et al., 1993b; Hurek et al., 1997b; Reinhold-Hurek and Hurek, 2000).

List of species of the genus Azospira

1. **Azospira oryzae** Reinhold-Hurek and Hurek 2000, 658[VP]
   o′ ry.zae. L. fem. *Oryza* genus name of rice; L. gen. n. *oryzae* from rice, referring to its frequent occurrence in association with rice roots.

   Description as for the genus with the following additional characteristics. Optimal growth at 40°C. Utilizes L-aspartate, L-glutamate, 3-hydroxybutyrate, isovalerate, and D-tartrate.

   The mol% G + C of the DNA is: 65.2.
   Type strain: 6a3, LMG9096.
   GenBank accession number (16S rRNA): AF011347.

Genus V. **Azovibrio** Reinhold-Hurek and Hurek 2000, 657[VP]

A.zo′ vi.bri.o. Fr. n. *azote* nitrogen; L. v. *vibrare* move rapidly to and fro, vibrate; M.L. masc. n. *Azovibrio* nitrogen-fixing organism which vibrates.

Gram-negative somewhat curved motile rods (0.6–0.8 × 1.5–3.6 µm). One polar flagellum. Chemoorganoheterotrophic and microaerophilic. Strictly respiratory; O_2 and nitrate are electron acceptors. Oxidase positive. **Fix N_2.** Grow on acetate, ethanol, fumarate, L-glutamate, DL-lactate, L-malate, propionate, and succinate.

The mol% G + C of the DNA is: 64–65.

Type species: **Azovibrio restrictus** Reinhold-Hurek and Hurek 2000, 657.

FURTHER DESCRIPTIVE INFORMATION

Azovibrio restrictus does not form the elongated cells seen in stationary cultures of *Azospira oryzae* and *Azonexus fungiphilus* (Reinhold-Hurek and Hurek, 2000).

Azovibrio restrictus S5b2 was isolated from the roots of Kallar grass collected in Pakistan (Reinhold-Hurek et al.,1993b). Other strains were isolated from roots of *Oryza sativa* and from fungal sclerotia found in soil on which rice was grown.

Analysis of 16S rDNA sequences placed *Azovibrio* in the *Betaproteobacteria*; in these analyses, *Azovibrio* was most closely related to members of the genera *Thaurea* and *Rhodocyclus*. *Azovibrio* strains could be separated from *Azoarcus, Azospira,* and *Azonexus* strains by comparisons of fatty acid composition, SDS-soluble protein electrophoretic patterns, and BOX-PCR genomic fingerprinting. DNA–DNA hybridization results supported these divisions (Hurek et al., 1997b; Reinhold-Hurek and Hurek, 2000).

ENRICHMENT AND ISOLATION PROCEDURES

Enrichment and isolation procedures are described in Reinhold et al. (1986).

Maintenance and long-term storage procedures are described in Reinhold-Hurek et al. (1993b).

DIFFERENTIATION OF THE GENUS *AZOVIBRIO* FROM OTHER GENERA

Reinhold-Hurek and Hurek (2000) provide tables of characteristics that differentiate the genera *Azoarcus, Azonexus, Azospira,* and *Azovibrio* from each other and from *Acetobacter diazotrophicus, Burkholderia vietnamensis,* the genus *Azospirillum,* and the genus *Herbaspirillum.*

TAXONOMIC COMMENTS

This genus was first described as *Azoarcus* sp. group C (Reinhold-Hurek et al., 1993b; Hurek et al., 1997b; Reinhold-Hurek and Hurek, 2000).

List of species of the genus Azovibrio

1. **Azovibrio restrictus** Reinhold-Hurek and Hurek 2000, 657[VP]
   re′ stric.tus. L. adv. *restrictus* limited, restricted, referring to the restricted spectrum of carbon sources used for growth.

   Description as for the genus with the following additional characteristic: grows on L-aspartate.
   The mol% G + C of the DNA is: 64.8 (T_m).
   Type strain: S5b2.
   GenBank accession number (16S rRNA): AF011346.

Genus VI. **Dechloromonas** Achenbach, Michaelidou, Bruce, Fryman and Coates 2001, 531[VP]

THE EDITORIAL BOARD

De.chlo.ro.mo′ nas. L. pref. *de* from; Gr. adj. *chloros* green (chlorine); Gr. fem. n. *monas* unit, monad; N.L. fem. n. *Dechloromonas* a dechlorinating monad.

Gram-negative, nonsporeforming, facultatively anaerobic, motile rods (0.5×2 µm). Strictly respiratory metabolism. **Chlorate and perchlorate reduced to chloride**; organic electron donors. O_2 also serves as an electron acceptor; some strains use nitrate as an electron acceptor.
   The mol% G + C of the DNA is: 63.5.
   Type species: **Dechloromonas agitata** Achenbach, Michaelidou, Bruce, Fryman and Coates 2001, 531.

FURTHER DESCRIPTIVE INFORMATION

Dechloromonas agitata was isolated from paper plant pulp sludge (Achenbach et al., 2001).

Dechloromonas agitata can use acetate, butyrate, fumarate, malate, propionate, succinate, yeast extract, Fe(II), sulfide, and reduced 2,6-anthrahydroquinone as electron donors; it does not use benzene, benzoate, citrate, Casamino acids, citrate, ethanol, formate, glucose, hexadecane, hydrogen, methanol, *N*-octane, palmitate, phenol, or toluene. Electron acceptors include chlorate, perchlorate, and oxygen (Bruce et al., 1999).

Analysis of 16S rDNA sequences of *Dechloromonas agitata* and ten other isolates placed them in the *Betaproteobacteria* and showed that they are allied to the genera *Rhodocyclus* and *Azoarcus* (Achenbach et al., 2001).

ENRICHMENT AND ISOLATION PROCEDURES

Enrichment cultures were prepared using environmental samples suspended and incubated in anaerobic liquid medium that contains 10 mM acetate as electron donor and 10 mM chlorate as electron acceptor (Bruce et al., 1999; Coates et al., 1999). Pure cultures can be achieved using the same medium and the agar-shake serial dilution method described by Pfennig and Biebl (1981).

DIFFERENTIATION OF THE GENUS *DECHLOROMONAS* FROM OTHER GENERA

Dechloromonas agitata can be distinguished from *Dechlorosoma suillum* by the ability of the former to use Fe(II), sulfide, and reduced 2,6-anthrahydroquinone sulfonate as electron donors for chlorate reduction (Achenbach et al., 2001).

List of species of the genus Dechloromonas

1. **Dechloromonas agitata** Achenbach, Michaelidou, Bruce, Fryman and Coates 2001, 531[VP]
   a.gi.ta′ ta. L. fem. part. adj. *agitata* agitated, highly active.

   Description as for the genus with the following additional characteristics. Electron donors include acetate, butyrate, fumarate, malate, propionate, succinate, yeast extract, Fe(II), sulfide, and reduced 2,6-anthrahydroquinone. Electron acceptors include chlorate, perchlorate, and oxygen. Growth optima at 1% NaCl, pH 7.5, and 35°C.
   The mol% G + C of the DNA is: 63.5 (HPLC).
   Type strain: CKB, ATCC 700666, DSM 13637.
   GenBank accession number (16S rRNA): AF 047462.

Genus VII. **Dechlorosoma** Achenbach, Michaelidou, Bruce, Fryman and Coates 2001, 531[VP]

THE EDITORIAL BOARD

De.chlo.ro.so' ma. L. pref. *de* from; Gr. adj. *chloros* green (chlorine); Gr. neut. n. *soma* body; N.L. neut. n. *Dechlorosoma* dechlorinating body.

Gram-negative, nonsporeforming motile rods (0.3 × 1.0 μm). **Nonfermentative facultative anaerobes. Chlorate and perchlorate reduced to chloride; organic electron donors.** O_2 and nitrate also serve as electron acceptors.

The mol% G + C of the DNA is: 65.8.

Type species: **Dechlorosoma suillum** Achenbach, Michaelidou, Bruce, Fryman and Coates 2001, 532.

FURTHER DESCRIPTIVE INFORMATION

Dechlorosoma suillum strains were isolated from samples from a swine waste treatment lagoon (Coates et al., 1999).

Dechlorosoma suillum PS is able to grow with acetate, butyrate, ethanol, fumarate, isobutyrate, lactate, malate, pyruvate, succinate, valerate, and Casamino acids as electron donors; it does not use benzoate, catechol, citrate, formate, glucose, glycerol, methanol, or hydrogen as electron donors. Chlorate, nitrate, and oxygen can serve as electron acceptors for growth on acetate; 2,6-anthraquinone disulfonate, fumarate, malate, selenate, sulfate, Fe(III), and Mn(IV) cannot (Coates et al., 1999).

Analysis of 16S rDNA sequences of *Dechlorosoma suillum* PS and three other isolates placed them in the *Betaproteobacteria* and showed that they are allied to the genera *Rhodocyclus* and *Azoarcus* (Achenbach et al., 2001).

ENRICHMENT AND ISOLATION PROCEDURES

Enrichment cultures can be prepared using environmental samples suspended and incubated in anaerobic liquid medium that was 10 mM in both acetate and chlorate as electron donor and acceptor, respectively (Bruce et al., 1999; Coates et al., 1999). Pure cultures can be achieved using the same medium and the agar-shake serial dilution method described by Pfennig and Biebl (1981).

DIFFERENTIATION OF THE GENUS *DECHLOROSOMA* FROM OTHER GENERA

Dechlorosoma suillum can be distinguished from *Dechloromonas agitata* by the ability of the latter to use 2,6-anthrahydroquinone sulfonate, Fe(II), and sulfide as electron donors for chlorate reduction (Achenbach et al., 2001).

List of species of the genus Dechlorosoma

1. **Dechlorosoma suillum** Achenbach, Michaelidou, Bruce, Fryman and Coates 2001, 532[VP]
   su.il' lum. L. neut. adj. *suillum* pertaining to swine.

   Description as for the genus with the following additional characteristics. Electron donors include acetate, butyrate, Casamino acids, ethanol, lactate, and propionate.

   Electron acceptors include chlorate, nitrate, oxygen, and percholorate. Reduce nitrate to N_2. Growth optima at 0% NaCl, pH 6.5, and 37°C.

   The mol% G + C of the DNA is: 75.8 (HPLC).
   Type strain: PS, ATCC BAA-33, DSM 13638.
   GenBank accession number (16S rRNA): AF 170348.

Genus VIII. **Ferribacterium** Cummings, Caccavo, Spring and Rosenzweig 2000, 1953[VP] (Effective publication: Cummings, Caccavo, Spring and Rosenzweig 1999, 187)

THE EDITORIAL BOARD

Fer' ri.bac.te' ri.um. L. neut. n. *ferrum* iron; Gr. neut. n. *bacterion* a small rod; M.L. neut. n. *Ferribacterium* rod-shaped iron bacterium.

Strictly anaerobic straight or curved rods (0.3–0.5 × 1.4–2.0 μm); contain poly-β-hydroxybutyrate granules. Nonsporeforming. **Oxidizes acetate, benzoate, formate, and lactate using Fe(III) as the electron acceptor**; also oxidizes acetate using fumarate, or nitrate as electron acceptors.

The mol% G + C of the DNA is: unknown.

Type species: **Ferribacterium limneticum** Cummings, Caccavo, Spring and Rosenzweig 2000, 1953 (Effective publication: Cummings, Caccavo, Spring and Rosenzweig 1999, 187.)

FURTHER DESCRIPTIVE INFORMATION

Ferribacterium limneticum CdA-1 was isolated from a sediment core taken in Lake Coeur d'Alene, Idaho, USA (Cummings et al., 1999).

Ferribacterium limneticum CdA-1 is a strict anaerobe that grows only when Fe (III) is present in the medium; it does not grow in a complex organic medium. Electron donors are acetate, benzoate, formate, and lactate. Citrate, ethanol, glucose, isopropanol, methanol, propionate, and succinate are not used as electron donors. As (V), Mn (IV), Se (VI), nitrite, sulfate, sulfite, thiosulfate, and trimethylamine are not used as electron acceptors when acetate is the electron donor (Cummings et al., 1999).

An analysis of 16S rDNA sequences showed that *Ferribacterium limneticum* CdA-1 belongs to the class *Betaproteobacteria* (Cummings et al., 1999).

ENRICHMENT AND ISOLATION PROCEDURES

Enrichment in liquid medium was carried out under anaerobic conditions using acetate as the electron donor and amorphous ferric oxyhydroxide as the electron acceptor (Cummings et al., 1999). Incubation for four months resulted in blackening of the culture; the organism was recovered by streaking onto solid medium containing Fe (III) pyrophosphate as electron acceptor.

List of species of the genus *Ferribacterium*

1. **Ferribacterium limneticum** Cummings, Caccavo, Spring and Rosenzweig 2000, 1953[VP] (Effective publication: Cummings, Caccavo, Spring and Rosenzweig 1999, 187.)
   lim.ne′ ti.cum. L. neut. n. *limne* lake; N.L. adj. *limneticum* from a lake.

The description is the same as that of the genus.
The mol% G + C of the DNA is: unknown.
Type strain: CdA-1.
GenBank accession number (16S rRNA): Y17060.

Genus IX. **Propionibacter** Meijer, Nienhuis-Kuiper and Hansen 1999, 1042[VP]

Theo A. Hansen

Pro.pioni.bac′ ter. M.L. n. *acidum propionicum* propionic acid; M.L. masc. n. *bacter* equivalent of Gr. neut. n. *bakterion* small rod; M.L. masc. n. *Propionibacter* propionic acid rod.

Rods 0.9–1.1 × 0.5–0.6 μm. Motile by means of a single polar flagellum. Multiply by binary fission. Gram negative. Do not form spores. **Anaerobic.** Can grow in stationary cultures under an air atmosphere, but not in well-aerated cultures. In the absence of nitrate, **substrates are fermented to propionate and acetate**. Growth occurs on simple organic compounds (sugars, dicarboxylic acids, sugar alcohols, and aspartate). Nitrate is reduced to nitrite. **Utilize N₂ as nitrogen source.** Isolated from estuary mud.

The mol% G + C of the DNA is: 61.

Type species: **Propionibacter pelophilus** Meijer, Nienhuis-Kuiper and Hansen 1999, 1043.

Enrichment and Isolation Procedures

Propionibacter has been obtained by direct anaerobic isolation from black surface mud of the Ems-Dollard estuary at the border between the Netherlands and Germany. Roll tubes with aspartate medium are used (Hansen et al., 1990). Strain asp 66, the type strain of *Propionibacter pelophilus*, is the most numerous aspartate-fermenting bacterium.

Maintenance Procedures

DSM Medium 503 (FWM medium; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) supplemented with 0.2 g/l yeast extract and 20 mM aspartate or another suitable energy substrate can be used for routine cultivation. For long-term preservation, the organism can be stored in ampules in medium supplemented with 8% glycerol under liquid nitrogen or at −80°C.

Differentiation of the genus *Propionibacter* from other genera

Propionibacter is most closely related to *Propionivibrio* (Tanaka et al., 1990; Hippe et al., 1999) based on the 16S rDNA sequences (approximately 3% difference between the type species). Both genera have a (mainly) fermentative metabolism with propionate and acetate as characteristic products. *Propionibacter* cells are straight rods, whereas *Propionivibrio* consists of curved rods. *Propionibacter* uses a wider range of substrates than does *Propionivibrio*; the latter is restricted to C₄-dicarboxylic acids.

Characteristics that differentiate *Propionibacter* from other genera and species of bacteria that carry out propionic fermentation are listed in Table BXII.β.115.

Taxonomic Comments

In this edition of the *Manual,* the genus *Propionibacter* is placed in the class *Betaproteobacteria,* the order *Rhodocyclales,* and the family *Rhodocyclaceae.* Other genera included in this family are *Rhodocyclus, Azoarcus, Azonexus, Azospira, Azovibrio, Ferribacterium, Propionivibrio, Thauera,* and *Zoogloea.*

The phylogenetic positions of *Propionibacter* and *Propionivibrio* have become clear only recently (Hippe et al., 1999; Meijer et al., 1999). The small difference in 16S rRNA gene sequence (approximately 3%) and the similarity in fermentative patterns would support a possible inclusion of *Propionibacter* in *Propionivibrio*; this, however, would require an emendation of the genus *Propionivibrio* as described by Tanaka et al. (1990).[*]

List of species of the genus *Propionibacter*

1. **Propionibacter pelophilus** Meijer, Nienhuis-Kuiper and Hansen 1999, 1043[VP]
   pe.lo′ phi.lus. Gr. n. *pelos* mud; Gr. adj. *philus* loving; M.L. adj. *pelophilus* mud-loving.

   Characteristics are as described for the genus, with the following additional features. Gram-negative cell envelope ultrastructure with an outer membrane. Colonies on anaerobic plates with aspartate are slimy white. Best growth occurs under anoxic conditions in sulfide-reduced media. Growth occurs in mineral media with a single fermentable substrate.

   The following are utilized as energy sources: L-aspartate, fumarate, L-malate, pyruvate, oxaloacetate, citrate, fructose, glucose, xylose, gluconate, arabitol, xylitol, mannitol, arabinose, and mannose. The following substrates are not used:

L-alanine, L-serine, L-threonine, L-methionine, L-glutamate, L-histidine, L-arginine, L-lysine, lactate, α-ketoglutarate, glycolate, succinate, propionate, butyrate, glycerol, ethanol, propanol, 2,3-butanediol, acetoin, cellulose, cellobiose, lactose, sucrose, maltose, melibiose, starch, sorbose, rhamnose, galactose, sorbitol, pectin, peptone, xylan, and inulin.

Indole and urease negative. Gelatin is not liquefied. Growth occurs on glucose and aspartate at pH 6.5–8.5 (optimal pH 7.5–8.0). The final pH of glucose-fermenting cul-

Editorial Note: While this volume of the *Manual* was in preparation, Brune et al. (2002) published a valid proposal to transfer the type species of the genus *Propionibacter, Propionibacter pelophilus,* to the genus *Propionivibrio.* Since the genus *Propionibacter* was monospecific, bacteriologists adhering to the proposal recognize that the genus *Propionibacter* thereby ceased to exist.

TABLE BXII.β.115. Characteristics that differentiate the genus *Propionibacter* from other genera and species of anaerobic bacteria that carry out a propionate fermentation[a]

Characteristic	*Propionibacter*	*Pelobacter propionicus*	*Desulfobulbus propionicus*	*Propionibacterium*	*Propionivibrio*
Gram-staining reaction	−	−	−	+	−
Cell shape:					
Straight rods	+	+	−	+[b]	−
Curved rods	−	−	−	−	+
Ovoid, rod-shaped, or lemon-shaped	−	−	+	−	−
Sugars are fermented	+	−	−	+	−
N_2 can be fixed	+	+	+	−	
$SO_4{}^{2-}$ can be used as a terminal electron acceptor for anaerobic respiration	−	−	+	−	−

[a]For symbols, see standard definitions.

[b]Pleomorphic.

tures is pH 5.5. Sulfate can be used as a sulfur source; growth in the absence of sulfide leads to cell clumps.

Optimal temperature 27–30°C; no growth at 35°C. Habitat: anoxic freshwater and estuarine sediments.

The mol% G + C of the DNA is: 60.8 (T_m).

Type strain: asp 66, DSM 12018.

GenBank accession number (16S rRNA): AF016690.

Genus X. *Propionivibrio* Tanaka, Nakamura and Mikami 1991b, 331[VP] (Effective publication: Tanaka, Nakamura and Mikami 1990, 327) emend. Brune, Ludwig and Schink 2002, 443

THE EDITORIAL BOARD

Pro.pi.o.ni.vi' bri.o. M.L. n. *acidum propionicum* propionic acid; M.L. masc. n. *Vibrio* that which vibrates, a generic name; M.L. masc. n. *Propionivibrio* the propionic acid vibrio.

Gram-negative, curved or straight **rods**. **Motile by means of a single polar flagellum.** Do not form spores. **Strict anaerobe or aerotolerant.** Multiply by binary fission. Chemoorganotrophic. Propionate and acetate formed as end products of fermentation. Succinate decarboxylated to propionate. Mesophilic. Member of the class *Betaproteobacteria*.

The mol% G + C of the DNA is: 60.8–61.6.

Type species: **Propionivibrio dicarboxylicus** Tanaka, Nakamura and Mikami 1991b, 331 (Effective publication: Tanaka, Nakamura and Mikami 1990, 327.)

List of species of the genus Propionivibrio

1. **Propionivibrio dicarboxylicus** Tanaka, Nakamura and Mikami 1991b, 331[VP] (Effective publication: Tanaka, Nakamura and Mikami 1990, 327.)

   di.car.bo.xy' li.cus. M.L. adj. *dicarboxylicus* pertaining to dicarboxylic acid.

   Gram-negative, curved to helical rods with tapered and rounded ends, 0.5–0.6 × 1.0–2.0 μm. Chains are helical. Motile in an active tumbling motion by means of a single polar flagellum. Catalase negative. Do not reduce nitrate, sulfate, sulfite, thiosulfate, or S^0. Utilize maleate, fumarate, and L-malate as carbon and energy sources, forming propionate and acetate as end products. Succinate decarboxylated to propionate. Do not utilize acetoin, acrylate, corn oil, crotonate, formate, glycerol, H_2/CO_2/acetate, lactate, peptone, pyruvate, yeast extract, monosaccharides, disaccharides, or trisaccharides. Optimal temperature 30°C (range 26–34°C); no growth at 20°C or 40°C. Optimal pH 6.7 (range 6.2–7.1). Habitat: anaerobic mud of freshwater sediments.

   The mol% G + C of the DNA is: 61.

   Type strain: CreMal1, DSM 5885, JCM 7784.

   GenBank accession number (16S rRNA): Y17601.

2. **Propionivibrio limicola** Brune, Ludwig and Schink 2002, 443[VP]

   li.mi' co.la. L. n. *limus* mud; L. v. *colere* to inhabit; N.L. n. *limicola* living in mud.

   Gram-negative; straight, slender rods, 0.6–0.7 × 1.5–2.5 μm. Motile by single polar flagella. Oxidase and catalase negative; superoxide dismutase positive. Do not form spores. Chemoorganotrophic. Fermentative metabolism, utilizing quinic acid and shikimic acid and producing acetate, propionate, and CO_2. No external electron acceptors utilized. Does not utilize sugars, alcohols, carboxylic acids, amino acids, or aromatic compounds. Aerotolerant; grows in unreduced media when incubated under air without agitation. Optimal temperature: 37°C; no growth at 45°C. pH range: 6.0–8.0; optimal pH: 7.0–7.5. Optimal growth in freshwater media. Growth inhibited in brackish media with 10 g/l NaCl and 1.0 g/l $MgCl_2$. Habitat: anoxic freshwater sediments.

   The mol% G + C of the DNA is: 61.6 ± 2.

   Type strain: GolChi1, ATCC BAA-290, DSM 6832.

   GenBank accession number (16S rRNA): AJ307983.

3. **Propionivibrio pelophilus** (Meijer, Nienjuis-Kuiper and Hansen 1999) Brune, Ludwig and Schink 2002, 444[VP] (*Propionibacter pelophilus* Meijer, Nienhuis-Kuiper and Hansen 1999, 1043.)*

pe.lo' phi.lus. Gr. n. *pelos* mud; Gr. adj. *pelophilus* mud-loving.

Gram-negative cell envelope ultrastructure with an outer membrane. Colonies on anaerobic plates with aspartate are slimy white. Best growth occurs under anoxic conditions in sulfide-reduced media. Growth occurs in mineral media with a single fermentable substrate. The following are utilized as energy sources: L-aspartate, fumarate, L-malate, pyruvate, oxaloacetate, citrate, fructose, glucose, xylose, gluconate, arabitol, xylitol, mannitol, arabinose, and mannose.

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*Editorial Note: This description of *Propionivibrio pelophilus* is that of *Propionibacter pelophilus* and has been reproduced here from the preceding chapter because of a proposal to transfer *Propionibacter pelophilus* to the genus *Propionivibrio.*

The following substrates are not used: L-alanine, L-serine, L-threonine, L-methionine, L-glutamate, L-histidine, L-arginine, L-lysine, lactate, α-ketoglutarate, glycolate, succinate, propionate, butyrate, glycerol, ethanol, propanol, 2,3-butanediol, acetoin, cellulose, cellobiose, lactose, sucrose, maltose, melibiose, starch, sorbose, rhamnose, galactose, sorbitol, pectin, peptone, xylan, and inulin. Indole and urease negative. Gelatin is not liquefied. Growth occurs on glucose and aspartate at pH 6.5–8.5 (optimal pH 7.5–8.0). The final pH of glucose-fermenting cultures is pH 5.5. Sulfate can be used as a sulfur source; growth in the absence of sulfide leads to cell clumps. Nitrate reduced to nitrite. N_2 utilized as nitrogen source. Optimal temperature 27–30°C; no growth at 35°C. Habitat: anoxic freshwater and estuarine sediments.

The mol% G + C of the DNA is: 60.8.

Type strain: asp 66, CIP 106101, DSM 12018.

GenBank accession number (16S rRNA): AF016690.

Genus XI. **Thauera** Macy, Rech, Auling, Dorsch, Stackebrandt and Sly 1993, 139[VP] emend. Song, Young and Palleroni 1998, 893

JOHANN HEIDER AND GEORG FUCHS

Thau' e.ra. M.L. fem. n. *Thauera* named after R.K. Thauer, a German microbiologist.

Rods 0.5–1.4 × 1.4–3.7 μm, usually occurring singly. Some species exhibit great variability of cell form, from rods to coccoid forms of different sizes. Gram negative. **Most species are motile.** No resting stages known. **Oxidase positive.** Catalase positive. Colonies on minimal medium are nonpigmented. **Aerobic,** having a strictly respiratory type of metabolism; never fermentative. **Molecular oxygen, nitrate, nitrite, and nitrous oxide are used as terminal electron acceptors,** and the nitrogen oxides are reduced to N_2O or N_2; one species also uses selenate, which is reduced to elemental Se. Optimal temperature 28–40°C. All species grow at pH 7.5; pH optima of different species range from 7.2 to 8.4. **Chemoorganotrophic, using various organic acids, amino acids, and aromatic and aliphatic compounds** as sole substrates. Only a **few carbohydrates are utilized**. Ammonium and nitrate salts can be used as sole nitrogen sources. **No N_2 fixation** known. Not proteolytic on casein or gelatin. Starch, cellulose, chitin, and agar are not hydrolyzed. Occur in polluted freshwater and wet soil environments and wastewater treatment plants.

The mol% G + C of the DNA is: 64–69.

Type species: **Thauera selenatis** Macy, Rech, Auling, Dorsch, Stackebrandt and Sly 1993, 140.

FURTHER DESCRIPTIVE INFORMATION

Cell morphology and fine structure Cells of *Thauera* spp. are usually rods 0.5–1.0 μm in width and 1.4–3.4 μm in length. Variation in cell size is commonly observed in liquid cultures. Most strains have been found to accumulate poly-β-hydroxybutyrate granules in the late growth phase. Flagella are usually present; flagellar insertion in the described species is either polar monotrichous or peritrichous with up to eight flagella; cells of some strains have either no flagella or a single flagellum inserted at any point, which has been termed "degenerately peritrichous" (Song et al., 1998). No sheaths, capsules, or other extracellular features are known.

Colonial and cultural characteristics A red-brown pigmentation is observed in densely packed cells or in colonies on solid medium, which is due to normal cell constituents, e.g., ferredoxins or cytochromes. Therefore, *Thauera* spp. can be classified as nonpigmented. Exceptions are the production of large amounts of insoluble red elemental selenium by *T. selenatis* growing under selenate-reducing conditions and the production of an as yet uncharacterized red pigment (metabolic intermediate or side product) by *T. aromatica* strain AR-1 growing anaerobically on α-resorcylate in the presence of nitrate (Gallus et al., 1997).

Lipid composition Major fatty acids contained in membrane lipids are *cis*-9-hexadecenoate ($C_{16:1\ \omega7c}$), hexadecanoate ($C_{16:0}$), and *cis*-11-octadecenoate ($C_{18:1\ \omega7c}$). Relative amounts of these fatty acids in different species are 41–58%, 19–32%, and 10–16%, respectively. Less abundant fatty acids found in amounts of <8% in all species are dodecanoate ($C_{12:0}$) and 3-hydroxydecanoate ($C_{10:0\ 3OH}$). Small amounts (0.7–2.1%) of a cyclic fatty acid ($C_{17:0\ cyclo}$) produced in the stationary growth phase have been reported as a characteristic property of *T. aromatica* and *T. chlorobenzoica* strains, whereas they are lacking in *T. selenatis*, *T. linaloolentis*, *T. terpenica*, and *Azoarcus* spp. (Song et al., 2000a, 2001).

Nutrition and growth conditions *Thauera* species grow in mineral medium with ammonium ions or nitrate as the nitrogen source and simple organic compounds as sole carbon and energy sources. Only a few species require organic growth factors. All species are chemoorganotrophic and obligately respiratory, requiring either oxygen or an alternative final electron acceptor, such as nitrate, nitrite, nitrous oxide, or selenate. The optimal growth temperature for most species is around 30°C, but one species has an optimal of 40°C.

Metabolism and metabolic pathways All species are obligately respiratory, but can shift between aerobic respiration and denitrification (dissimilatory nitrate reduction to N_2O or N_2). One species is capable of anaerobic respiration on selenate. The first step, reduction of selenate to selenite, is catalyzed by a unique periplasmic selenate reductase, an enzyme containing a molybdenum cofactor, iron–sulfur clusters, and a cytochrome *b*

(Schröder et al., 1997; Krafft et al., 2000). Three of the known species catabolize various aromatic compounds anaerobically under denitrifying conditions. Two other species catabolize terpenoids, but not aromatic compounds, in the absence of oxygen. Whereas not much is known about the biochemistry involved in terpenoid metabolism, the anaerobic metabolism of aromatic substrates has been well studied. All tested species can catabolize some aromatic compounds under aerobic conditions. The aromatic substrates utilized aerobically and under denitrifying conditions are not necessarily the same, and the presence of completely different pathways for the same substrates has been demonstrated for *T. aromatica* and the physiologically similar *Azoarcus evansii* (Ziegler et al., 1989; Altenschmidt et al., 1993; Heider et al., 1998; Mohamed, 2000). Many organic acids and several amino acids serve as sole substrates aerobically and anaerobically for all species, whereas most species are very limited in their capacities to catabolize sugars.

Metabolism of aromatic compounds   Aromatic substrates that are utilized under anaerobic conditions by strains of *T. aromatica* include benzoate, 3- and 4-hydroxybenzoate, phenol, toluene, phenylacetate, 4-hydroxyphenylacetate, and halogenated benzoates. Most of these substrates are first converted via diverse peripheral metabolic pathways to a common intermediate, benzoyl-CoA (Heider and Fuchs, 1997a, b). Benzoyl-CoA is then further catabolized by a conserved metabolic pathway, as shown in Fig. BXII.β.108. The first step is a two-electron reduction to a non-aromatic derivative, cyclohexa-1,5-diene-1-carboxy-CoA, by the key enzyme benzoyl-CoA reductase. This enzyme catalyzes benzoyl-CoA reduction, coupling this reaction to the hydrolysis of two moles of ATP (Boll et al., 1997). Further metabolic steps proceed via a beta-oxidation-like pathway, which leads to the first open-chain intermediate 3-hydroxypimelyl-CoA (Fig. BXII.β.108), generated by hydrolytic ring opening (Laempe et al., 1998, 1999). The benzoyl-CoA reductase of several analyzed *T. aromatica* strains appears to be immunologically distinct from the enzyme of closely related *Azoarcus* strains (Mechichi et al., 2002). The intermediate 3-hydroxypimelyl-CoA is degraded to three acetyl-CoA units and one CO$_2$, and acetyl-CoA is finally oxidized to CO$_2$ via the tricarboxylic acid cycle. Anaerobic catabolism of some aromatic compounds, such as α-resorcylate, apparently does not occur via the benzoyl-CoA intermediate. The first steps of a completely different pathway have recently been described for *T. aromatica* strain AR-1 that leads to oxidation of α-resorcylate to the intermediate hydroxyhydroquinol (Gallus and Schink, 1998). Catabolism of other aromatic substrates by strain AR-1 appears to proceed via the benzoyl-CoA pathway (Philipp and Schink, 2000). All tested *Thauera* species catabolize aromatic compounds aerobically. Aromatic substrates used under aerobic conditions include benzoate, phenylacetate, and 2-aminobenzoate. The catabolic pathways used for aerobic catabolism of aromatic compounds differ from those used under denitrifying growth conditions and involve the participation of oxygenases in all known instances.

Habitats   Members of the genus *Thauera* are common saprophytic inhabitants of polluted freshwater and wet soil environments. They occur in wastewater treatment plants and are apparently naturally enriched in groundwater aquifers, river, lake, and pond sediments that are contaminated with aromatic or aliphatic organic compounds or toxic inorganic compounds such as selenate. They seem to be involved in mineralization and detoxification of xenobiotic contaminants in these habitats.
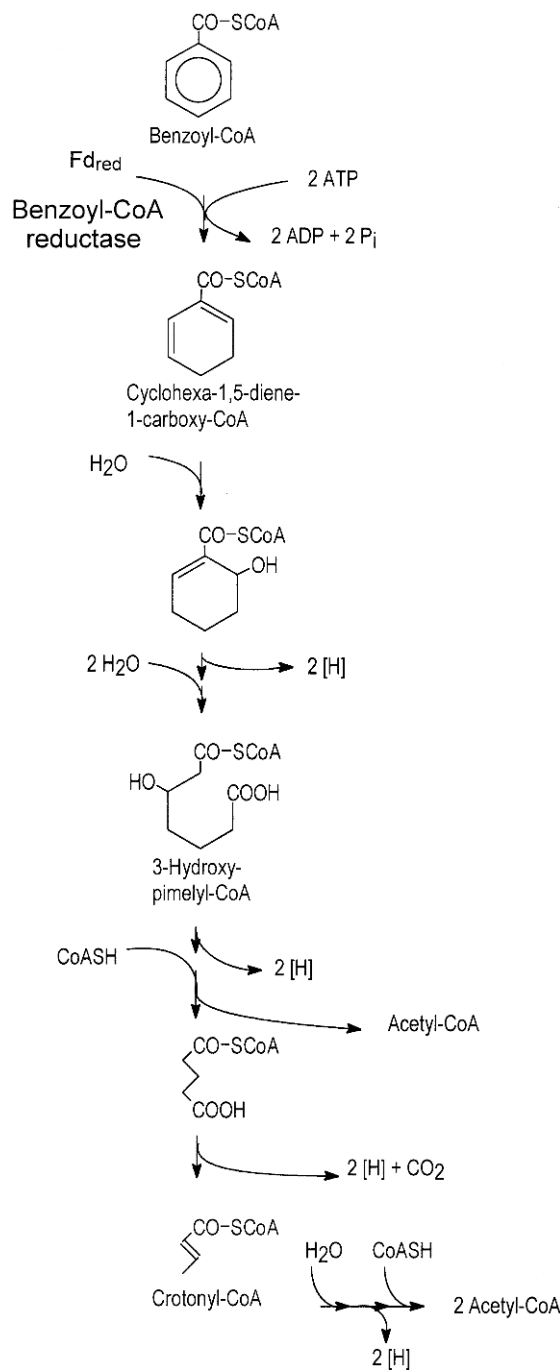

FIGURE BXII.β.108.   Anaerobic benzoyl-CoA catabolic pathway of *T. aromatica*. Benzoate is converted to benzoyl-CoA by a coenzyme A ligase (AMP forming). For other substrates converted to this intermediate, see Heider and Fuchs (1997a, b).

Pathogenicity   No pathogenic or symbiotic associations are known with plants or animals.

ENRICHMENT AND ISOLATION PROCEDURES

There is no selective isolation procedure for *Thauera* strains. The known species have been obtained from enrichments designed for special physiological properties that are shared by strains of various other taxa. For example, anaerobic catabolism of aromatic compounds is also found in the related genus *Azoarcus* and

even in some less related recent isolates of denitrifying *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* (Spormann and Widdel, 2000), and the anaerobic catabolism of terpenoids has also been reported for an *Alcaligenes* species (Foss et al., 1998a). *Thauera* strains are commonly isolated from wastewater treatment plants or contaminated soil or freshwater habitats after enrichment under anaerobic respiratory conditions. The enrichments are performed under mesophilic conditions at pH values of 7–8 in a buffered mineral salt medium containing either nitrate or selenate as the electron acceptor. Organic acids, aromatic compounds, or terpenoids may be used as sole substrates. Growth on some phenolic compounds or on acetone requires CO_2 or bicarbonate in the medium. Pure cultures are obtained after serial dilution in agar shakes under denitrifying conditions. However, highly purified agar (e.g., Oxoid purified agar) should be used for growth on solid media, because several strains are known to be sensitive to impurities in the agar. Alternative solidifying agents such as Gelrite should be tested to improve growth of some strains.

MAINTENANCE PROCEDURES

Stock cultures of *Thauera* strains grown under denitrifying conditions can be maintained in liquid cultures at 4°C for up to 3 months. Cultures of *Thauera* strains can be preserved for years in liquid nitrogen if they are supplemented with 5% (v/v) dimethylsulfoxide.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

No specific 16S rRNA targeted probes have yet been derived for members of the genus *Thauera*. However, such oligonucleotide probes have been designed for *in situ* detection of strains of the closely related genus *Azoarcus* (Hess et al., 1997). Since no cross-reaction with *T. aromatica* has been recorded with these probes, it should be possible to design analogous probes for *Thauera* species. Another potentially useful procedure for characterizing the *Thauera* species that are capable of anaerobic catabolism of aromatic compounds is the immunological detection of benzoyl-CoA reductase. Antibodies raised against purified benzoyl-CoA reductase from the type strain of *T. aromatica* specifically react with extracts of several different strains of *Thauera* sp., but do not react with any of several tested *Azoarcus* spp. grown anaerobically on benzoate (Mechichi et al., 2002).

DIFFERENTIATION OF THE GENUS *THAUERA* FROM OTHER GENERA

The most distinctive characteristics of *Thauera* are the ability to grow on unusual organic substrates and the limited ability to catabolize carbohydrates. These properties are shared with *Achromobacter*, *Acidovorax*, *Alcaligenes*, *Bordetella*, *Burkholderia*, *Comamonas*, *Pseudomonas*, *Ralstonia*, and *Zoogloea* species, as well as with the closely related genus *Azoarcus*. Useful phenotypic properties to discriminate these species are listed in Table BXII.β.116. The currently described species of the above-mentioned genera, except *Azoarcus*, are differentiated by their inability to degrade aromatic compounds such as benzoic acid in the absence of oxygen, but it can be expected that new isolates capable of anaerobic degradation of aromatic compounds will also be affiliated with some of these genera. *Azoarcus* strains are distinguished from *Thauera* sp. by their ability to fix dinitrogen and to use various sugars. Most above-mentioned genera belong to the class *Betaproteobacteria*, as does *Thauera*.

TAXONOMIC COMMENTS

The genus *Thauera* is defined mainly based on 16S rDNA phylogeny. The few criteria listed in Table BXII.β.116 to differentiate most species of the genus from physiologically similar genera (e.g., absence of N_2 fixation) are not tested for all known species, and it may be expected that *Thauera* species will be isolated that deviate from some of these criteria. The six validly described species have been obtained following very different enrichment procedures and vary considerably in their physiological properties.

Phylogeny within the genus is currently based on 16S rDNA and DNA–DNA reassociation data. As shown in Fig. BXII.β.109, a phylogenetic tree of all known 16S rRNA sequences of *Thauera* strains currently in the database shows that the genus is monophyletic and well separated from *Azoarcus* spp., the closest known relatives. The positions of the *T. terpenica* and *T. linaloolentis* strains justify their treatment as separate species. The 16S rDNA sequences of the other four described species, *T. selenatis*, *T. aromatica*, *T. chlorobenzoica*, and *T. mechernichensis* are highly similar to one another, but treatment as separate species is supported by mutual DNA–DNA reassociation values that are significantly lower than 70% (Song et al., 1998, 2000a, 2001; Scholten et al., 1999). These four species apparently form a subcluster of related species within the genus (Fig. BXII.β.109). An additional strain, ATCC 700265, is included in the species *T. aromatica*, as justified by the observed similarity (99.2% 16S rRNA identity and 77% DNA–DNA reassociation; Song et al., 1998). Recently, a three-dimensional representation of the DNA–DNA hybridization values among *Thauera* strains has indicated that *T. chlorobenzoica* ATCC 700723[T], which is capable of degrading halobenzoates under denitrifying conditions, clusters together with additional isolates that are similarly capable of degrading halobenzoates. As a result, these strains have been separated from *T. aromatica* and placed into a separate species, *T. chlorobenzoica* (Song et al., 2001).

A number of recently isolated bacterial strains also belong to the genus *Thauera* based on their 16S rDNA sequences, but have not yet been fully described. Several of these strains belong to the species *T. aromatica*. One of these, strain mXyN1, which was isolated under denitrifying conditions with *m*-xylene as the sole carbon and energy source (Rabus and Widdel, 1995), exhibits 99.7% 16S rRNA identity with the type strain and 90% relatedness by DNA–DNA reassociation (Scholten et al., 1999). Another strain, AR-1 (DSM 11528), is capable of growing anaerobically on α-resorcylate and shares 99.9% 16S rDNA identity with the type strain (Gallus et al., 1997). Several other strains with 16S rDNA sequence identities of 99 ± 0.1% with the *T. aromatica* type strain have been characterized in a survey of halobenzoate-degrading denitrifying bacteria; these strains are 3CB-2, 3CB-3, 3BB-1, 4FB-1, and 4FB-2 in Fig. BXII.β.109 (Song et al., 2000b). Strains 4FB-1, 4FB-2, and 3BB-1 were later reclassified as reference strains of *T. chlorobenzoica*, and strains 3CB-2 and 3CB-3 were reclassified as reference strains of *T. aromatica*, on the basis of DNA–DNA hybridization studies (Song et al., 2001). The 16S rDNA sequences of two other recently characterized strains cluster between those of *T. selenatis* and *T. chlorobenzoica* (Fig. BXII.β.109). One of these, strain mz1t, was recently isolated directly from a bacterial floc of activated sludge using a micromanipulator (Lajoie et al., 2000), the other, strain PN-1, was isolated 30 years ago from a denitrifying enrichment culture with 4-hydroxybenzoate (Taylor et al., 1970). Strain PN-1 had been classified as *Achromobacter xylosoxidans* by physiological properties (Blake and Hegeman, 1987) and has only recently been iden-

TABLE BXII.β.116. Characteristics differentiating the genus *Thauera* from other bacterial genera[a]

Characteristics	*Thauera*	*Azoarcus*	*Zoogloea*	*Acidovorax*	*Comamonas*	*Burkholderia*	*Alcaligenes*	*Ralstonia*	*Achromobacter*	*Bordetella*	*Pseudomonas*
Flagella	1–8	1	1	1	>1	>1	1–8	1–4	1–8	1–8	1 or >1
Animal pathogens in genus	–	–	–	–	–	–	+	–	+	+	+
Plant pathogens in genus	–	–	–	–	–	+	–	+	–	–	+
Plant symbionts in genus	–	+	–	–	–	–	–	–	–	–	–
Saprophytes in genus	+	+	+	+	+	+	+	+	+	–	+
Denitrification	+	+[b]	+	–	–	–[c]	+	+	+	D	D
Urease	–	–[d]	D		–		–	–		+	
Growth factor requirement	D	–	–	–	–	–		–	D	+	–
Autotrophic growth with H$_2$	–[e]	–	–	+	–	–	–	D	D	–	–
Aerobic growth on:											
Glucose/fructose	–	+[b]	D	+	–	+	–	D	D		+
Ribose	–	–	D	+	–	+	–	D	–		+
Glutarate	+	+		+	+	+	D	+			+
Adipate	D	D		+	+	+	–	+	+		D
Pimelate	–	D		D	+	+	–	+	+		–
Ethanol	D	–[b]	+	–	D	+					D
4-Hydroxybenzoate	+	D		–	+	+	–	+	–	–	+
Benzoate	+	+[b]	+	–	D	+					D
Phenylacetate	+	D		–	D	+	+	D	D		–
Anaerobic growth with aromatic substrates	D	D	–	–	–	–	–	–	–	–	–
N$_2$ fixed	–	+[f]	–	–	–	–	–	–	–	–	–
Poly-β-hydroxybutyrate accumulation	+	+	+	D	+	+	D	+	+		–
Mol% G +C of DNA	64–69	62–67	65	62–69	63–69	67	56–60	64–68	64–70	69	58–70
Class:											
Betaproteobacteria	+	+	+	+	+	+	+	+	+	+	
Gammaproteobacteria											+

[a]Symbols: see standard definitions. Blank space, data not available or not applicable.

[b]Not for plant-symbiotic species.

[c]Nitrate reduced to nitrite.

[d]Urease present in *A. indigens*.

[e]Autotrophy has been described in *T. selenatis*.

[f]Not all strains tested.

tified as a *Thauera* sp. based on its 16S rRNA sequence (GenBank AF170281). Two other strains of *Thauera* sp., strains O and 1917, isolated from an anoxic leachate treatment reactor, are apparently less related to the species clustering with *T. aromatica*, as indicated by branching of their 16S rDNA sequences before branching of the *T. linaloolentis* sequence (Fig. BXII.β.109; Etchebehere et al., 2001a). Strain O has been reported to degrade benzoate under denitrifying conditions (Etchebehere et al., 2001a), but there is no more information available on the physiology of these strains. Finally, a number of bacterial strains isolated under denitrifying conditions on different aromatic substrates (strains S100, LG356, SP, B4P, and S2; Tschech and Fuchs, 1987; Seyfried et al., 1991) have also been found to be *Thauera* spp. on the basis of >98% 16S rDNA sequence identity with the *T. aromatica* type strain. New species have recently been established for two of these strains, *T. aminoaromatica* (strain S2) and *T. phenylacetica* (strain B4P), while the others were described as

further reference strains of *T. aromatica* (for details, see Mechichi et al., 2002).

Nomenclatural problems within the genus *Thauera* arise mainly from the fact that very high degrees of 16S rDNA identity are accompanied by wide physiological variability between the species and even between several strains of one species. Therefore, taxonomy cannot be based on 16S rDNA sequences alone, but rather needs to be complemented by DNA–DNA reassociation studies and the physiological properties of the strains. For example, the establishment of *T. aromatica* and *T. selenatis* as separate species was much more dependant on the profound physiological differences between the strains than on the highly similar 16S rRNA sequences, and this speciation is corroborated by low DNA–DNA reassociation values (Anders et al., 1995). From the speed of description of new *Thauera* isolates in the last few years, it may be expected that the number of strains and species in the genus will expand rapidly in the future.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *THAUERA*

Characteristics useful for the differentiation of the species of the genus *Thauera* are listed in Tables BXII.β.117 and BXII.β.118.

List of species of the genus Thauera

1. **Thauera selenatis** Macy, Rech, Auling, Dorsch, Stackebrandt and Sly 1993, 140[VP]

se.le.na′ tis. M.L. gen. n. *selenatis* of selenate, according to the electron acceptor used for isolation.
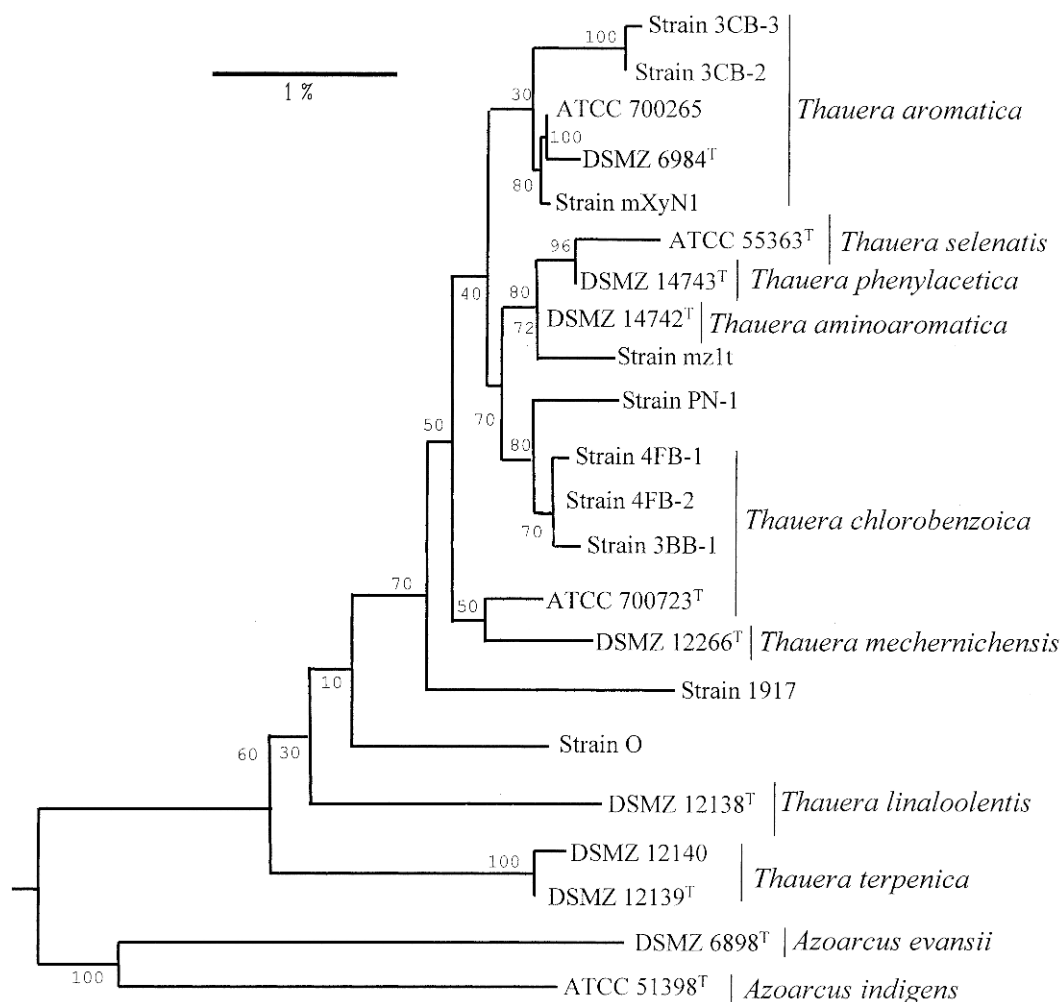
FIGURE BXII.β.109. Phylogenetic tree of the 16S rRNA sequences of bacterial strains affiliated with the genus *Thauera* and the type strains of *Azoarcus evansii* and *Azoarcus indigens*. All sequences larger than 1 kb currently in the database were included. The tree was derived from a multiple alignment of a segment of 1070 bases present in all sequences (bases 177–1244 in 16S rDNA of *T. aromatica* DSM 6984[T]) by the neighbor-joining method. Bootstrapping values of the branch points are indicated. Currently affiliated species names are indicated to the right of the strain designations. Strain ATCC 700723 has recently been reclassified as the type strain of the species *T. chlorobenzoica*. Similarly, strains 4FB-1, 4FB-2, and 3BB-1 have been reclassified as reference strains of *T. chlorobenzoica*, and strains 3CB-2 and 3CB-3 as reference strains of *T. aromatica* (Song et al., 2001). Accession numbers for 16S rDNA sequences of not validly described strains, which are not included in the list of species are: strain mXyN1, X83533; strain mz1t, AF110005; strain PN-1, AF170281; strain 1917, AJ277705; strain O, AJ277704. Accession numbers of *T. aminoaromatica* and *T. phenylacetica* are AJ315677 and AJ315678, respectively. Note that database entry Y17591 was used for the 16S rRNA of *T. selenatis*.

TABLE BXII.β.117. Characteristics differentiating the species of the genus *Thauera*[a]

Characteristic	*T. selenatis*	*T. aromatica*	*T. chlorobenzoica*	*T. linaloolentis*	*T. mechernichensis*	*T. terpenica*
Flagellation:						
Monotrichous	+	−	−	−	+	+
Peritrichous	−	+	+	−	−	−
Optimal pH	8	7.2–7.8	7.5–8.0	7.0–7.3		7.9–8.8
Optimal temperature, °C	28	28–37	30–37	32	40	32
SeO_4^{2-} can be used as a terminal electron acceptor	+	−	−	−	−	−
Mol% G + C of DNA	66	67	69	66	65	64

[a]Symbols: see standard definitions. Blank space, data not reported or not applicable.

TABLE BXII.β.118. Growth of *Thauera* species on various substrates under aerobic (O_2) and denitrifying (NO_3^-) conditions[a]

Substrate	*T. selenatis* O₂	NO₃⁻	*T. aromatica* O₂	NO₃⁻	*T. chlorobenzoica* O₂	NO₃⁻	*T. linaloolentis* O₂	NO₃⁻	*T. mechernichensis* O₂	NO₃⁻	*T. terpenica* O₂	NO₃⁻
Autotrophic on H_2/CO_2	+			−								
Benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate	+	−	+	+	+	+		−	+	+		−
3-Aminobenzoate			−	+	−	+						
Toluene			−	+	−	−		−				−
Phenol			−	D	−	−			−	+		
Phenylacetate, phenylalanine	+		+	+				−	+	+		−
4-Hydroxyphenylacetate			+	+	−	−						
Benzyl alcohol			−	−	−	+						
Vanillate			+	+	+	+						
2-Fluorobenzoate, 4-fluorobenzoate			D	D	+	+						
3-Chlorobenzoate, 3-bromobenzoate, 3-iodobenzoate			−	D	−	D						
Linalool, geraniol								+				−
Terpenes (e.g., menthol, eucalyptol)								−				+
Ethanol			D	+	−	D		+				−
Formate	+			−				−				−
Acetate, butyrate	+	+	+	+	+	+		+	+	+		+
Caproate			D	D	D	+		+				+
Propionate	+		+	+	−	−		+				+
Isobutyrate	+		+									
Glutarate			+	+				−				+
Adipate			D	D	D	D						
Pimelate			−	−								
Succinate, fumarate	+		+	+	+	+		+				+
Aspartate	+		−	−								
Glutamate	+		+	+	+	+		+	+	+		+
Proline	+		−	−								
Leucine	+		+	+					+	+		
Valine			−	−				+				+
Serine	+		D	D								
Alanine			D	D	−	−						
β-Alanine	+		D	−								
Glucose, fructose, ribose, lactose	+		−	−					−			−

[a]Symbols: see standard definitions. Blank space, data not reported or not applicable.

Characteristics are as described for the genus and as listed in Tables BXII.β.117 and BXII.β.118. Additional features are as follows. Cells possess a single polar flagellum. Selenate is reduced to selenite by a periplasmic selenate reductase, and the selenite is further reduced to elemental (red) selenium, which precipitates in the culture medium. No aromatic compounds are catabolized anaerobically. For further descriptive information see Macy et al. (1993). Optimal temperature, 28°C. Isolated from selenate-contaminated water by use of enrichment cultures under selenate-reducing conditions.

The mol% G + C of the DNA is: 66 (T_m).

Type strain: AX, ATCC 55363.*

GenBank accession number (16S rRNA): X68491, Yl7591.

2. **Thauera aromatica** Anders, Kaetzke, Kämpfer, Ludwig and Fuchs 1995, 331[VP]

a.ro.ma′ti.ca. M.L. adj. *aromatic* referring to the nutritional preferences of this organism.

Editorial Note: The type strain of *Thauera selenatis* is currently unavailable as it was deposited in association with a patent application that was subsequently abandoned.

Characteristics are as described for the genus and as listed in Tables BXII.β.117 and BXII.β.118. Additional features are as follows. Cells may have peritrichous flagellation with up to eight flagella or may have lost all but one flagellum ("degenerately peritrichous"). Benzoate and various other aromatic compounds are catabolized anaerobically via the benzoyl-CoA pathway. Selenate is not reduced. Optimal temperature, 28°C. Isolated from (contaminated) water and soil, particularly from denitrification stages of sewage treatment plants. Enrichment is possible under denitrifying conditions with aromatic substrates. Different isolates vary in their catabolic capacities for aromatic compounds.

The mol% G + C of the DNA is: 66–67 (HPLC).

Type strain: K172, DSM 6984.

GenBank accession number (16S rRNA): X77118.

Additional Remarks: The accession numbers for reference strains ATCC 700265, CB-2, and CB-3 are U59176, AF229881, and AF229882, respectively.

3. **Thauera chlorobenzoica** Song, Palleroni, Kerkhof and Häggblom 2001, 600[VP]

chlor.o.ben.zo′i.ca. M.L. adj. *chloro* pertaining to chlorine; from Gr. adj. *chloros* pale green; M.L. adj. *benzoicus* pertaining to benzoic acid; M.L. fem. adj. *chlorobenzoica* indicating the ability to utilize chlorobenzoic acid.

Characteristics are as described for the genus and as listed in Tables BXII.β.117 and BXII.β.118. Additional features are as follows. Cells are 0.85–0.97 × 1.2–2.7 μm and have peritrichous flagellation. In contrast to *T. aromatica*, all known strains of the species grow under denitrifying conditions on 2- and 4-fluorobenzoate; some strains also grow on 3-chlorobenzoate, 3-bromobenzoate, or 3-iodobenzoate. Isolated from river and estuarine sediments and from agricultural soil.

The mol% G + C of the DNA is: 69 (HPLC).

Type strain: 3CB-1, ATCC 700723.

GenBank accession number (16S rRNA): AF123264.

Additional Remarks: The accession numbers for reference strains 4FB-1, 4FB-2, and 3BB-1 are AF229867, AF229868, and AF229887, respectively.

4. **Thauera linaloolentis** Foss and Harder 1999, 2^VP (Effective publication: Foss and Harder 1998, 370.)
li.na.lo.o.len′tis. Sp. n. *linaloe* wood of American trees, from which an oil containing mainly linalool is extracted; L. *olere* smell; M.L. adj. *linaloolentis* linaloe-smelling, referring to the ability of the organism to catabolize linalool.

Characteristics are as described for the genus and as listed in Tables BXII.β.117 and BXII.β.118. Additional features are as follows. Catabolizes some open-chain terpenoids, such as linalool or geraniol, anaerobically. No anaerobic catabolism of benzoate or other aromatic compounds occurs. For further descriptive information, see Foss and Harder (1998). Isolated from activated sewage sludge after enrichment for denitrifying bacteria with linalool as the sole substrate. Optimal temperature, 32°C.

The mol% G + C of the DNA is: 66 (HPLC).

Type strain: 47Lol, DSM 12138.

GenBank accession number (16S rRNA): AJ005816.

5. **Thauera mechernichensis** Scholten, Lukow, Auling, Kroppenstedt, Rainey and Kiekmann 1999, 1049^VP
me.cher.ni.chen′sis. M.L. adj. *mechernichensis* pertaining to Mechernich, Germany, the site of isolation of the organism.

Characteristics are as described for the genus and as listed in Tables BXII.β.117 and BXII.β.118. Additional features are as follows. The cells have single polar flagella. Anaerobic catabolism of benzoate and other aromatic compounds occurs. Denitrification is not repressed under aerobic conditions. For further descriptive information, see Scholten et al. (1999). Isolated from the nitrification step of a leachate treatment plant after aerobic continuous culture with acetate as the carbon source. Optimal temperature, 40°C.

The mol% G + C of the DNA is: 65 (HPLC).

Type strain: TL1, DSM 12266.

GenBank accession number (16S rRNA): Y17590.

6. **Thauera terpenica** Foss and Harder 1999, 2^VP (Effective publication: Foss and Harder 1998, 372.)
ter.pe′ni.ca. M.L. adj. *terpenica* related to terpenes, referring to the nutritional preferences of these organisms.

Characteristics are as described for the genus and as listed in Tables BXII.β.117 and BXII.β.118. Additional features are as follows. Catabolize several monoterpenes anaerobically. The substrate range of catabolized terpenes varies for different isolates. For example, the terpenoid substrates used by strain DSM 12139^T include eucalyptol, sabinene, β-pinene, α-phellandrene, and other chemically similar compounds, whereas those used by strain DSM 12140 include menthol, isomenthol and some similar compounds. No anaerobic catabolism of benzoate or other aromatic compounds occurs. For further descriptive information, see Foss and Harder (1998). Isolated from forest ditches after enrichment culture for denitrifying bacteria with defined monoterpenes as sole substrates. Optimal temperature, 32°C.

The mol% G + C of the DNA is: 64 (HPLC).

Type strain: 58Eu, DSM 12139.

GenBank accession number (16S rRNA): AJ005817.

Additional Remarks: The accession number for the 16S sequence for strain DSM 12140 is AJ005818. The recently described *p*-cymene degrading bacterial strain pCyN2 (Harms et al., 1999a) may also belong to this species based on its very similar 16S rDNA sequence (accession number Y17285).

Other Organisms

While this volume was in press, two more *Thauera* species were validly published, *T. aminoaromatica* (DSM 14742) and *T. phenylacetica* (DSM 14743). For further details, see Mechichi et al. (2002). Furthermore, an aerobic butane-degrading bacterium previously desgnated as *"Pseudomonas butanovora"* was recently shown to be affiliated with *T. linaloolentis* on the basis of its 16S rDNA sequence, but has not yet been formally described as a new *Thauera* species (Anzai et al., 2000).

Genus XII. **Zoogloea** *Itzigsohn 1868, 39^AL emend. Shin, Hiraishi and Sugiyama 1993, 830*

Rɪᴄʜᴀʀᴅ F. Uɴᴢ

Zo.o.gloe′a. Gr. adj. *zoos* living; Gr. n. *gloia* glue; M.L. fem. n. *Zoogloea* living glue.

The following description is representative of the genus based on well-characterized isolates recovered directly from naturally occurring, typical, fingered zoogloeae: **Straight to slightly curved, plump rods**, 1.0–1.3 × 2.1–3.6 μm, with rounded ends; sometimes tapered to a blunt point at one or both poles. Nonsporeforming and noncystforming. **Cells in older cultures are demonstrably encapsulated.** Gram negative. **Actively motile**, especially in young cultures, by means of a **single polar flagellum. Intracellular granules of poly-β-hydroxybutyrate are formed** on media containing the salts of organic acids. Cultures enter into formation of flocs and films in liquid media at late growth stages; **the cells become embedded in gelatinous matrices to form zoo-**

gloeae, which may be distinguished by a "tree-like" or "finger-like" morphology. Young colonies on solid media under a normal air atmosphere are translucent and punctiform, but may increase to 1 or 2 mm in diameter and exhibit opaque centers. Nonpigmented. **Aerobic, having a strictly respiratory type of metabolism with oxygen or nitrate as the terminal electron acceptor. Denitrification occurs with formation of N_2.** Optimal temperature for growth, 28–37°C. Optimal pH, 7.0–7.5. **Oxidase positive.** Weakly catalase positive. Chemoorganotrophic. **Acid is not formed from carbohydrates except for xylose, glycerol, and ethanol, which are attacked oxidatively by a few strains.** Proteolytic on gelatin. Most strains are urease positive. Litmus milk is unchanged. Hydrogen sulfide is usually not produced from cysteine. Major carbon sources include salts of several organic acids (e.g., lactate, pyruvate, and fumarate), dicarboxylic amino acids (e.g., aspartate, glutamate, and asparagine), alcohols, and salts of certain aromatic acids (e.g., benzoate and *m*-toluate). Benzene derivatives are attacked by *meta* cleavage of the ring structure. Organic nitrogen compounds (e.g., dicarboxylic amino acids) and ammonia serve as nitrogen sources; nitrate is unsuitable. Specific growth factor requirements, if any, are unknown. Major cellular fatty acids are palmitoleic ($C_{16:1}$) and 3-hydroxydecanoic ($C_{10:0\ 3OH}$) acids. Major respiratory quinones are Q-8 and RQ-8. The major polyamine is 2-hydroxyputrescine. **Occur free-living in organically polluted fresh waters and in wastewaters at all stages of treatment.**

The mol% G + C of the DNA is: 67.3–69.0.

Type species: **Zoogloea ramigera** Itzigsohn 1868, 30.

FURTHER DESCRIPTIVE INFORMATION

Zoogloea strains form flocculent masses of zoogloeae in both complex and defined media containing suitable carbon sources (Fig. BXII.β.110). Arrangement of the bacteria into sharply demarcated columns or "fingers" (Fig. BXII.β.111), which protrude from a cluster or aggregate of cells, constitutes the historically recognized growth form of *Z. ramigera* (Koch, 1877; Butterfield, 1935; Dugan and Lundgren, 1960; Unz and Farrah, 1976b). Cells are embedded in an extracellular matrix, which is discernible by treatment of wet mount specimens with a contrasting agent (Fig. BXII.β.112) or by scanning electron microscopy (Fig. BXII.β.113). However, taken by itself, *Zoogloea* morphology is an unreliable character upon which to base the identification of *Z. ramigera*, since (a) fragmented portions of flocs and pellicles and artifacts created by random coalescence of bacteria may be mistaken under microscopic observation for finger-like zoogloeae, and (b) *Z. ramigera* may form amorphous, rather than finger-like, zoogloeae (Fig. BXII.β.114). The extent of zoogloea production varies among strains and with the culture conditions and may diminish greatly or be lost, especially during frequent transfer of strains in rich culture media.

Cells of *Zoogloea* are plump rods that are motile by means of a single, monopolar flagellum (Fig. BXII.β.115). Chains of cells are rare. Cells in very old cultures may appear elongated.

Colonies produced on CY agar[1] are initially punctiform. After 3 or 4 days, the colonies reach 1 mm in diameter and appear circular, slightly raised, and translucent with opaque centers (Fig. BXII.β.116) or completely gray-white. Colony edges are entire or lobate. Mature colonies are distinctively tenacious and cohesive and may be lifted intact from the agar surface with a needle. Colonies develop poorly on ordinary nutrient agar.
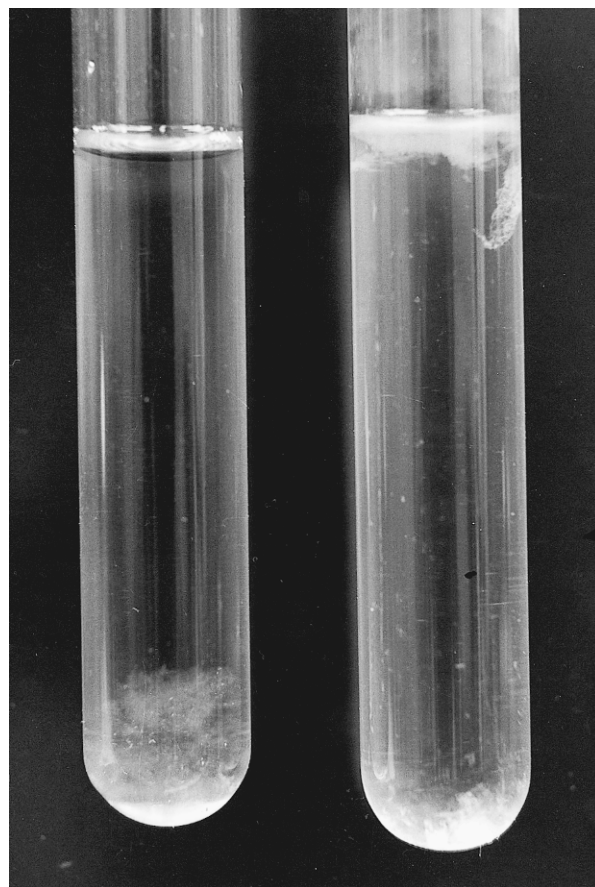

FIGURE BXII.β.110. Flocculent growth habit of *Zoogloea ramigera*. *Left*, ATCC strain 19544. *Right*, strain G4, freshly isolated and exhibiting the development of a thick, straggly, pellicle. Casitone–yeast autolysate medium, 28°C, 72 h.

Growth of *Zoogloea* strains is slow at 9°C and nonexistent at 45°C. The pH range for growth lies between pH 5.0 and 10.0, with poor development occurring at extreme pH values.

Strains survive, but do not grow, under strictly anaerobic conditions in the absence of nitrate. They exhibit a microaerophilic tendency in semisolid agar deeps, as evidenced by the appearance of culture bands 3–5 mm below the agar surface (Unz and Dondero, 1967b). Approximately 50% of strains tested reduce trimethylamine oxide to trimethylamine (Unz and Dondero, 1967b).

Acid was found to be produced oxidatively from xylose by 3 of 65 strains in one study (Unz and Dondero, 1967a), and by 5 of 37 strains in another study (Unz and Farrah, 1972). Acid was also found to be produced oxidatively from glycerol and/or ethanol by 10 of 65 strains (Unz and Dondero, 1967a).

No growth on citrate was found to occur in one study (Unz and Dondero, 1967a), but growth by 20 of 37 strains occurred on Koser's citrate in another study (Unz and Farrah, 1972).

Zoogloea strains are not fastidious nutritionally and may be cultured on a variety of organic carbon sources in a simple defined medium.[2] Carbon sources supporting growth of at least

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1. CY agar contains (g/l of distilled water): Casitone (Difco), 5.0; yeast autolysate, 1.0; agar, 15.0.

2. Defined medium (g/l distilled water): carbon source, 0.25–0.5; $(NH_4)_2SO_4$, 0.375; $MgSO_4·7H_2O$, 0.2; $CaCl_2$, 0.2; K_2HPO_4, 0.1; and $FeSO_4·7H_2O$, 0.005. The pH is adjusted to 7.2 with 0.1 N NaOH. Yeast autolysate (0.01 g) and vitamin B_{12} (1×10^{-6} g) may be included as sources of growth factors to decrease growth lag.
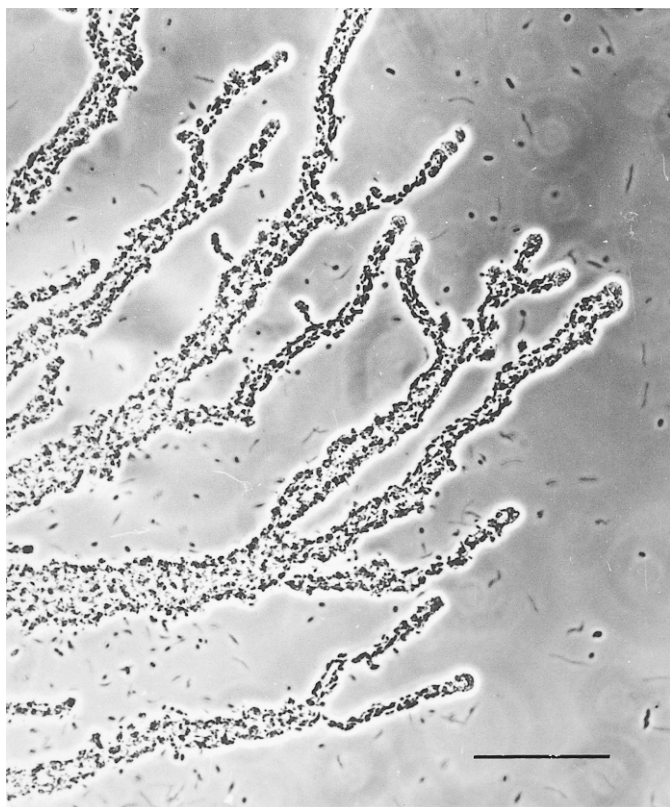
FIGURE BXII.β.111. Finger-like zoogloea. *Zoogloea ramigera* ATCC strain 19544 grown in lactate–mineral salts medium at 28°C for 60 h. Phase contrast. Bar = 50 μm.

90% of *Zoogloea* strains are lactate, pyruvate, α-hydroxybutyrate, fumarate, ethanol, butanol, benzoate, glutamate, aspartate, and asparagine (Unz and Dondero, 1967b).

Resistance to 2.5 U of penicillin G occurs in 67% of the strains tested (Unz and Dondero, 1967a).

Zoogloea strains are found principally in organically polluted fresh waters, wastewaters, and aerobic biological wastewater treatment systems, e.g., activated sludge and trickling filter units.

Enrichment and Isolation Procedures

Finger-producing strains of *Zoogloea* may be cultivated best in enrichment media inoculated with activated sludge or with the films from aerobic wastewater treatment devices. Approximately 2 ml of an inoculum are added to 15 ml of mineral salts solution[3] overlaying 5 ml of a nutrient-enriched agar[4] contained at the bottom of a metal-capped test tube (20 mm OD × 150 mm). The enrichment culture is incubated at 28°C until a pellicle develops, usually within 2 or 3 d if activated sludge is the inoculum. A simpler method for enrichment of fingered zoogloeae involves storage of activated sludge in covered glass containers at room temperature until a surface film appears (Amin and Ganapati, 1967). The latter method is not always reliable, and success may depend on obtaining a proper ratio of the height of activated sludge solids to the total volume of liquid stored in the container.

A wet mount of enrichment-culture surface film is examined at 100× magnification by phase contrast microscopy to confirm the presence of fingered zoogloeae. Several loopfuls of film are

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3. Mineral salts solution (g/l): $(NH_4)_2SO_4$, 0.3; NaCl, 5.85; $CaCl_2 \cdot 2H_2O$, 0.2; K_2HPO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.14; $FeSO_4 \cdot 7H_2O$, 0.0003; $MnCl_2 \cdot 4H_2O$, 0.0063; $CoSO_4 \cdot 7H_2O$, 0.0001; H_3BO_4, 0.0006; $ZnCl_2$, 0.00022; and $CuSO_4 \cdot 5H_2O$, 0.00008. The medium components are prepared from stock solutions and the pH of the medium is adjusted to 8.5 ± 0.1 pH unit with 0.5 N NaOH.

4. Nutrient-enriched agar: to mineral salts solution containing agar (20 g/l), any of the following sole carbon sources are added in the amount shown per liter: starch, 2.4 g; *m*-toluic acid (neutralized), 1.35 g; *n*-butanol, 1.5 ml; lactic acid (85%), 1.35 g; ethanol (95%), 1.5 ml; or glucose, 2.4 g. After adding the carbon source to the liquid mineral salts–agar mixture, the pH is adjusted to 8.5 ± 0.1 with 0.5 N NaOH.
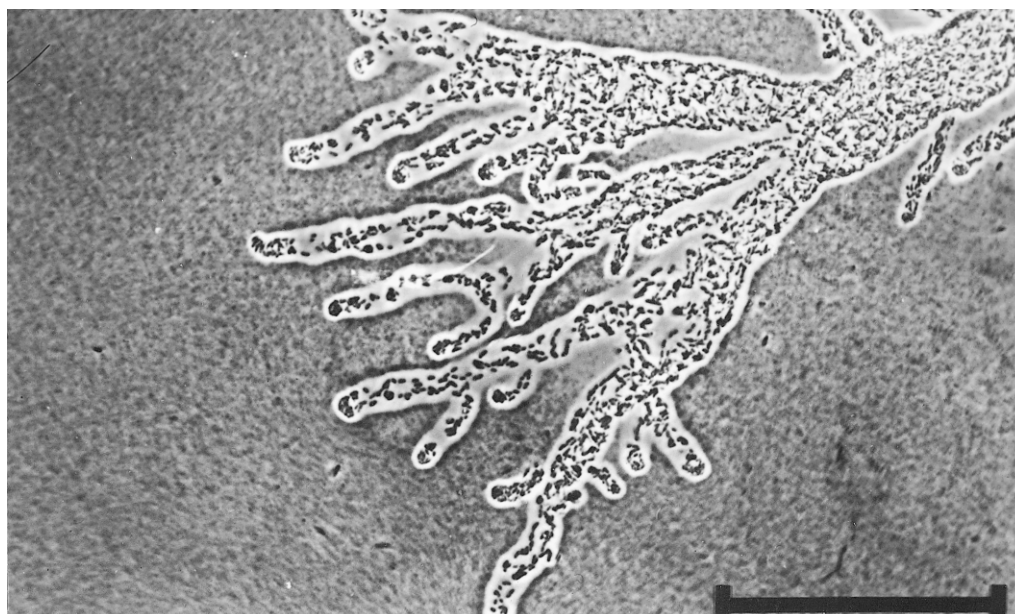

FIGURE BXII.β.112. Finger-like zoogloea treated in wet mount with skim milk to accentuate the exopolymer in which the cells are embedded. *Zoogloea ramigera* ATCC strain 19544. Lactate–mineral salts medium, 28°C, 60 h. Phase contrast. Bar = 100 μm. (Reproduced with permission of R.F. Unz, International Journal of Systematic Bacteriology *21:* 91–99, 1971 ©International Union of Microbiological Societies.)
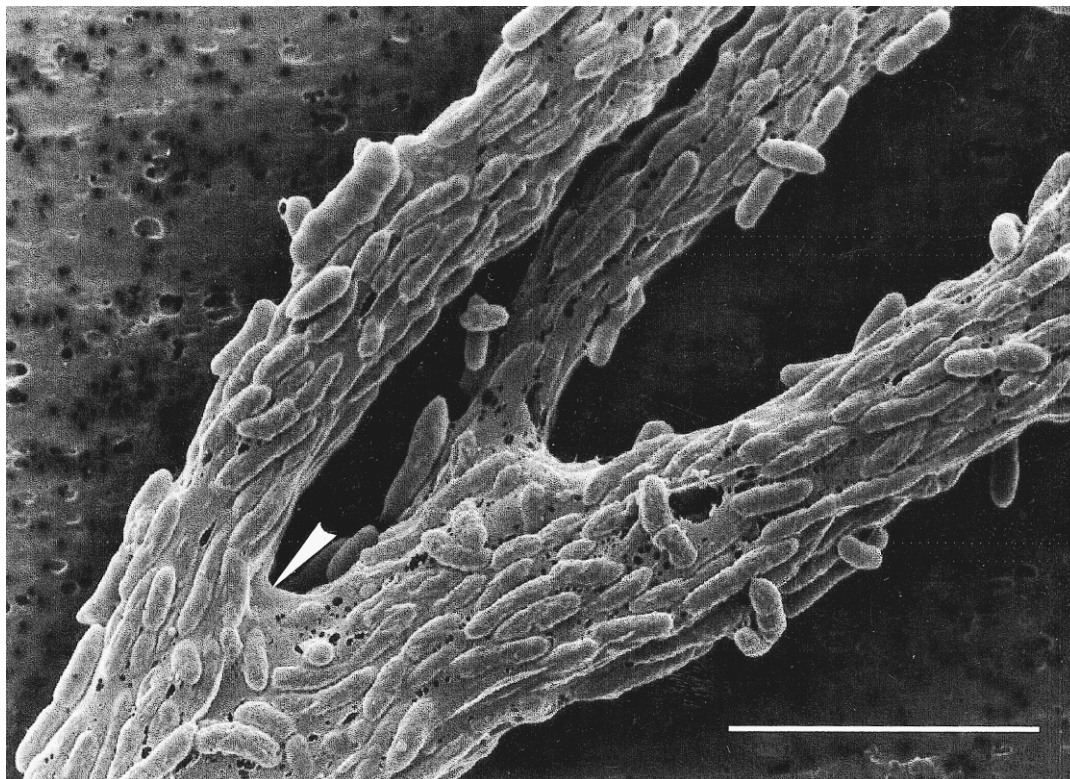
FIGURE BXII.β.113.   Finger-like zoogloea mounted on a nucleopore filter, dehydrated, fixed by gradient ethanol dehydration, washed with hexa-methyldisilizane, dried, sputter-coated with gold, and observed by field emission scanning electron microscopy. Cells embedded in a gelatinous matrix (*arrow*). *Zoogloea ramigera* ATCC 19544 grown in yeast extract (2.5 g/l)–peptone (2.5 g/l), 28°C. Bar = 7.2 μm.
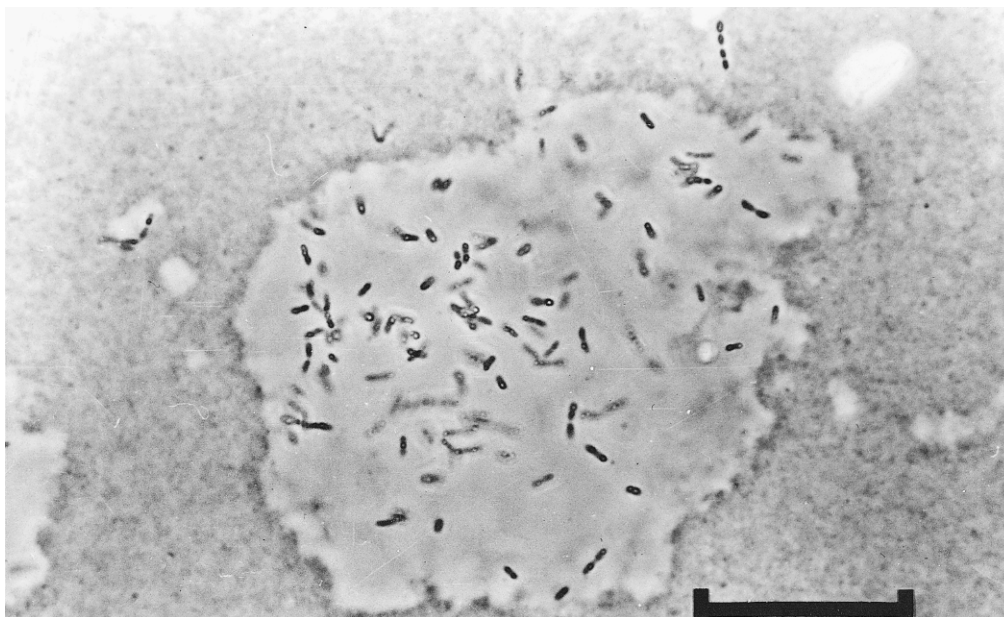

FIGURE BXII.β.114.   Amorphous zoogloea treated in wet mount with skim milk to accentuate the exopolymer in which the cells are embedded. *Zoogloea ramigera* ATCC strain 19544. Lactate–mineral salts medium, 28°C, 60 h. Phase contrast. Bar = 100 μm. (Reproduced with permission of R.F. Unz, International Journal of Systematic Bacteriology *21:* 91–99, 1971 ©International Union of Microbiologial Societies.)

transferred to a 2-ml droplet of CY broth contained in a Petri plate. The fingered zoogloeae may be located easily in the droplet under 45-60× magnification. Approximately 10–12 of the fin-gered zoogloeae are transferred individually and successively by a micropipette through each of four 0.7-ml droplets of CY me-dium in order to free loosely attached debris and microorganisms

FIGURE BXII.β.115. *Zoogloea ramigera* ATCC strain 19544 showing the single, polar flagellum. Casitone–yeast autolysate medium, 28°C, 24 h. Platinum–carbon shadowed. Bar = 1 μm. (Reproduced with permission of R.F. Unz, International Journal of Systematic Bacteriology *21:* 91–99, 1971 ©International Union of Microbiological Societies.)
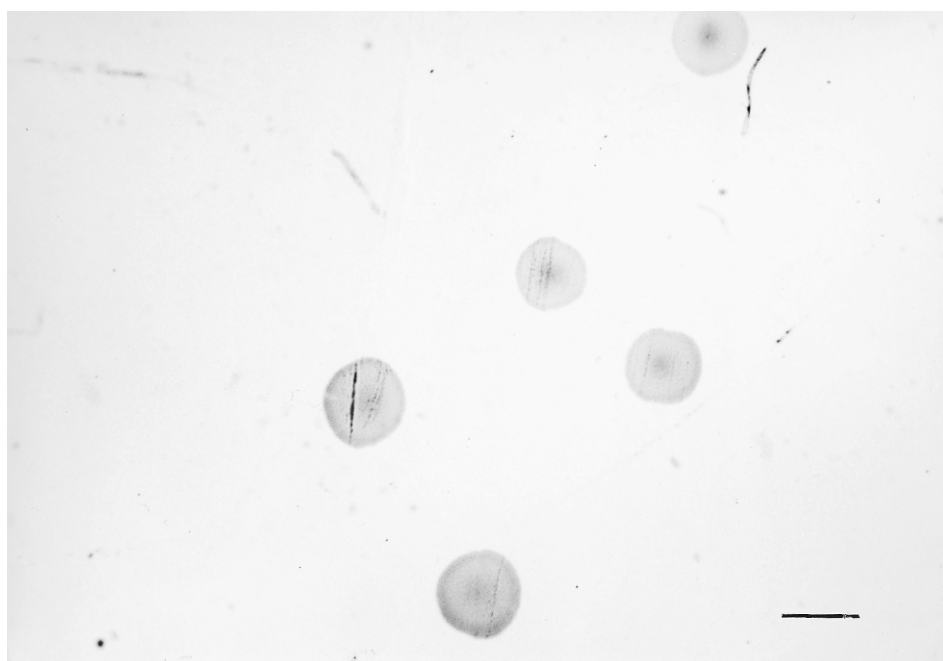
FIGURE BXII.β.116. Colonies of *Zoogloea ramigera* ATCC strain 19544. Casitone–yeast autolysate agar, 28°C, 72 h. Photographed by reflected light. Bar = 1 mm. (Reproduced with permission of R.F. Unz, International Journal of Systematic Bacteriology *21:* 91–99, 1971 ©International Union of Microbiological Societies.)

from the zoogloeae. Finally, the washed zoogloeae are collectively transferred to 3 ml of CY medium and subjected briefly to sonic oscillation (e.g., 30 s at 50 watts) to release the cells. A loopful of the sonicate is streaked onto solid CY medium and incubated at 28°C. Typically cohesive colonies of *Zoogloea* are large enough to be transferred intact to CY broth after 3–4 d. Inoculation of CY broth with a single colony usually results in the appearance of slight turbidity and a slippery, glistening pellicle after 3 d at

28°C. Pellicles may be composed entirely of fingered zoogloeae or amorphous zoogloeae, which, following detachment, settle and give rise to a flocculent sediment.

MAINTENANCE PROCEDURES

Zoogloea strains may be maintained in half-strength CY medium at 20°C for at least 2 months between subcultures. The formation of zoogloeal flocs is visibly reduced upon continuous transfer in
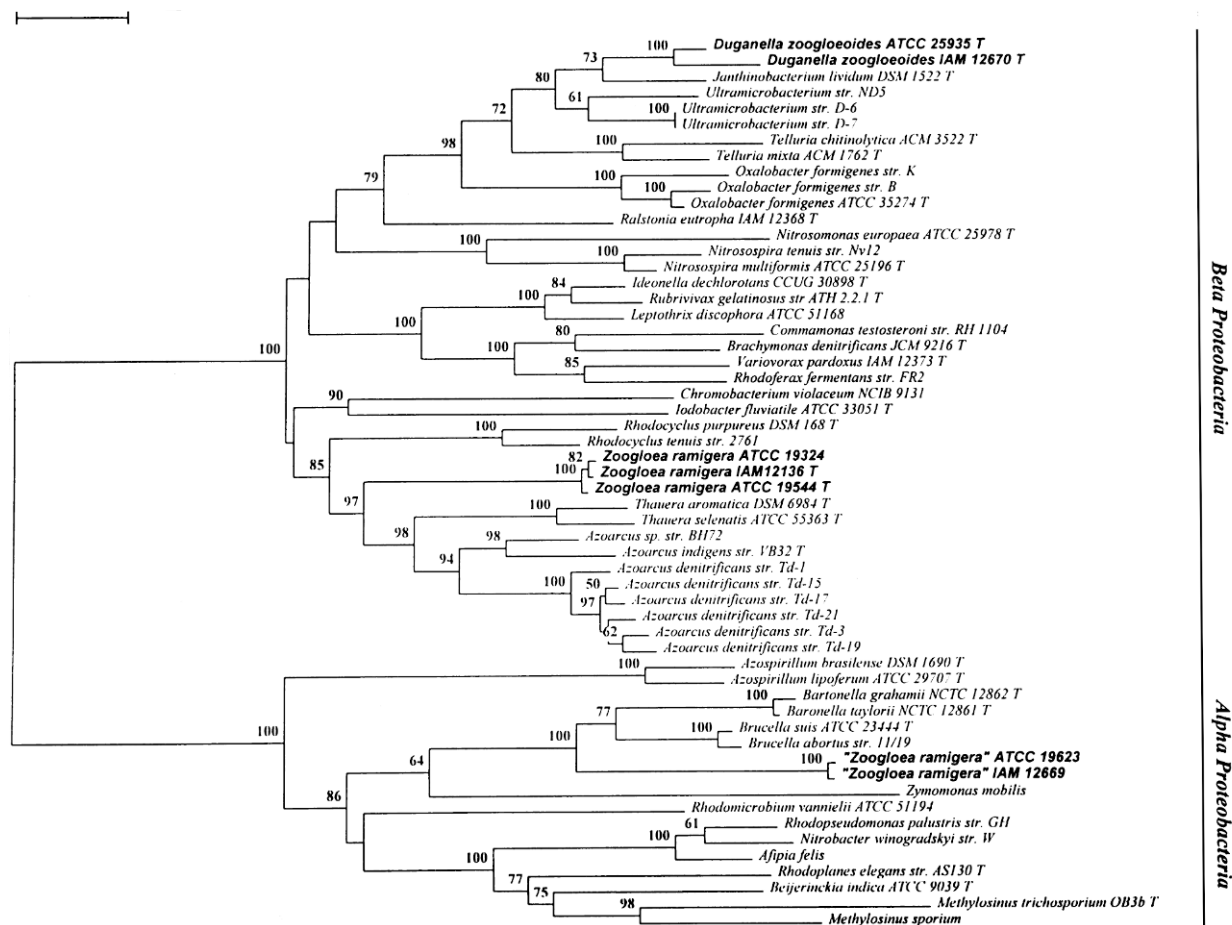
FIGURE BXII.β.117. Dendrogram revealing the phylogenetic position of *Zoogloea ramigera*. The phylogenetic distance tree was generated from 1309 bases of unambiguously aligned 16S rRNA sequences from 39 members of the class *Betaproteobacteria* and 17 members of the class *Alphaproteobacteria*. Positions corresponding to 33–68 plus 104–1376 (*Escherichia coli* numbering) were used. Phylogenetic distances were calculated with correction for multiple substitutions at a single position (Jukes and Cantor, 1969), and the tree was generated using the neighbor-joining method (Saitou and Nei, 1987), as implemented in the TREECON software package (Van de Peer and de Wachter, 1994). Bootstrap resampling (100 replicates) was used to indicate the confidence in the sequence clusters obtained. The values at nodes represent the percentage bootstrap support for the groupings to the right of the node. Bootstrap values below 50% are not shown. Bar = 2 nucleotide changes per 100 nucleotides in the 16S rRNA sequence (see text next page).

laboratory culture media. The zoogloeal growth habit may be restored by plating a broth culture and transferring a colony back to liquid medium.

Strains do not survive prolonged refrigeration; however, they may be preserved indefinitely by lyophilization or cryopreservation.

DIFFERENTIATION OF THE GENUS *ZOOGLOEA* FROM OTHER GENERA

The taxonomic status of the genus *Zoogloea* within the family *Pseudomonadaceae* has been called into question based on 16S rDNA sequence analyses and chemotaxonomic data (Shin et al., 1993). The most recent phylogenetic positioning of the type species of the genus *Zoogloea* lies within the *Betaproteobacteria* as shown in Fig. BXII.β.117. Inspection of the phylogenetic tree reveals that the three most closely related genera to the type strain of *Z. ramigera* (ATCC 19544) are *Thauera*, *Azoarcus*, and *Rhodocyclus*. Strains of authentic and misnamed *Z. ramigera* that have received molecular characterization are given in bold face. *Z. ramigera* ATCC 19324 was isolated by single-cell microdissection of a nat-

ural, finger-like zoogloea sampled from a trickling filter in a similar fashion as the type strain. Sequence data from independent studies of the type strain obtained from two different culture collections, namely *Z. ramigera* ATCC 19544 (Rosselló-Mora et al., 1993) and *Z. ramigera* IAM 12136 (Shin et al., 1993), are reflected in the dendrogram. Although mol% G + C of the DNA provides a basis for comparison, differentiation of the genus *Zoogloea* from the genera *Thauera*, *Azoarcus*, and *Rhodocyclus* is possible (Table BXII.β.119). Cell flocculation, which was historically held to be a distinctive feature of the genus *Zoogloea*, is widespread among eubacteria, including a species of the dinitrogen-fixing genus *Azoarcus* (Reinhold-Hurek et al., 1993b). The chemical composition of the exopolymers formed by organisms designated as *Zoogloea*, e.g., hexosamine, glucose, xylose, and arabinose (Crabtree et al., 1966); glucose, mannose, and galactose (Wallen and Davis, 1972); glucose and galactose (Parsons and Dugan, 1971); and glucosamine and fucosamine (Tezuka, 1973), is diverse and has not been useful in the taxonomic sense. The mucopolysaccharide (Farrah and Unz, 1976) produced by the neotype strain of *Z. ramigera* (ATCC 19544) appears to be chemically related to

TABLE BXII.β.119. Differential characteristics of the genus *Zoogloea* and phylogenetically related genera of the *Betaproteobacteria*[a]

| Characteristics	*Zoogloea*	*Azoarcus*[b]	*Rhodocyclus*[c]	*Thauera*[d]
Cell diameter typically 1.0 μm or slightly larger	+	−	−	−
Pigment formed by colonies	−	+	+	+
Photoautotrophic	−	−	+	−
Obligately chemooganotrophic	+	+	−	−
Denitrification	+	−[e]	−	+
Urease	+	D	D	
Oxidase	+	+		+
Catalase	w +[f]	+		+
Gelatinase	+	−	D	−
N$_2$ fixation	−	+	D	−
Poly-β-hydroxybutyrate	+			+
Predominant polyamine: 2-OH putrescine	+[g]			−
Mol% G + C of DNA	67.3–69.0[h]	62–68	64.8–72.4	66
Major respiratory quinones	Q-8, RQ-8[i]		Q-8, MK-8	Q-8

[a]For symbols see standard definitions.

[b]Data from Reinhold-Hurek et al. (1993b).

[c]Data from Imhoff and Trüper (1989).

[d]Data from Macy et al. (1993).

[e]Denitrification demonstrated by some strains (Hurek and Reinhold-Hurek (1995).

[f]Weakly positive.

[g]Data from Hamana and Matsuzaki (1993).

[h]Data from Shin et al. (1993).

[i]Data from Hiraishi et al. (1992c).

the exopolymer described by Tezuka (1973); moreover, it exhibits a fine, strand-like mesh (Fig. BXII.β.118), rather than the coarse, fibrillar network of cellulose-like glycans observed for other zoogloeal and nonzoogloeal bacteria (Friedman et al., 1968, 1969) or the cellulose of *Acetobacter* species (Ohad et al., 1962). The exocellular homo- and heteropolysaccharide exopolymers that have been characterized for species of *Xanthomonas*, *Pseudomonas*, *Arthrobacter*, and *Alcaligenes* are variably water-soluble and may increase the consistency of the culture medium (Powell, 1979; Sutherland, 1979a). In contrast, the mucopolysaccharide-producing neotype strain of *Z. ramigera* produces a water-insoluble exopolymer at room temperature and the culture medium is not visibly thickened.

The genus *Comamonas* (Davis and Park, 1962) has been used to classify non-floc-forming bacteria to distinguish them from otherwise similar floc-forming bacteria considered to be *Zoogloea* strains (Dias and Bhat, 1964).

TAXONOMIC COMMENTS

Important developments concerning the genus *Zoogloea* have occurred since the last edition of the *Manual* (Unz, 1984). These pertain to solidifying the position of ATCC 19544 (strain 106; Unz, 1971) as the neotype strain of the species *Z. ramigera* (Judicial Commission, 1979) and resolving the issue of two strains of uncertain affiliation. The type strain and ATCC 25935 (strain 115; Friedman and Dugan, 1968), both members of the class *Betaproteobacteria*, differ on the basis of the presence of rhodoquinone-8 and palmitoleic acid (major cellular fatty acid of the nonpolar fraction) in the former (Hiraishi et al., 1992c), putrescine and 2-hydroxyputrescine content (Hamana and Matsuzaki, 1993), and their 16S rRNA sequences (Rosselló-Mora et al., 1993). ATCC 19623 (strain I-16-M; Crabtree and McCoy, 1967) is confined to the class *Alphaproteobacteria* based on spermidine content (Hamana and Matsuzaki, 1993), ubiquinone Q-10 content (Hiraishi et al., 1992c), and 16S rDNA (Shin et al., 1993) and 16S rRNA (Rosselló-Mora et al., 1993) sequence analyses. The use of molecular biological techniques has resolved the am-
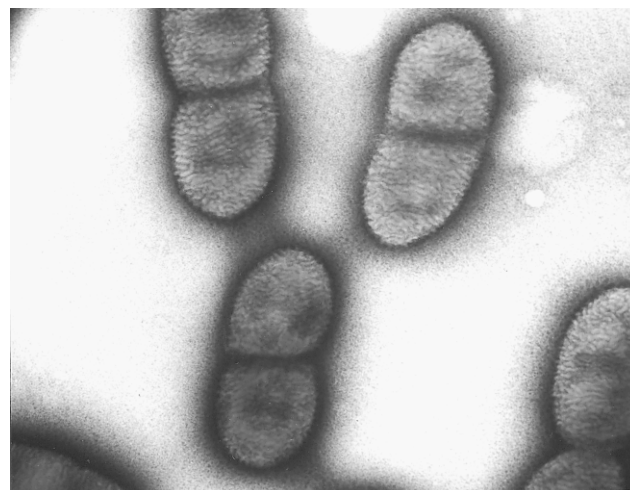
FIGURE BXII.β.118. Cells of *Zoogloea ramigera* ATCC strain 19544 embedded in a fine meshwork of exopolymer. Casitone–yeast autolysate medium, 28°C, 72 h. Preparation negatively stained with 2% phosphotungstic acid for 30 s × 27,000.

biguous taxonomic status of the genus *Zoogloea*, originally attributed to nomenclatural difficulties arising from (a) uncertainties about the type species, which was originally described on the basis of a growth form of an organism in mixed culture, (b) acceptance of the flocculent growth habit as the principal characteristic for the identification of sundry aggregative bacteria as strains of *Zoogloea* or of *Z. ramigera*, and (c) conflicting descriptions of bacteria stated to be *Z. ramigera*.

Z. ramigera ATCC 19544 is one of 147 *Zoogloea* strains of certain origin, in that the bacteria were isolated as single cells during micromanipulation of natural, finger-like zoogloeae under microscopic observation (Unz and Dondero, 1967a). *Z. ramigera* ATCC 19544 forms fingered zoogloeae in liquid culture, which

are similar in appearance to those of the natural type from which it came and to the zoogloeae referred to as "tree-like" ramifications of spherical gelatinous masses in the original description of the species (Itzigsohn, 1868). Recent evidence of a relationship of the type strain to cells in natural, finger-like zoogloeae is provided in the exclusive hybridization between the bacteria of natural zoogloeae and an oligonucleotide probe complementary to a region of 16S rRNA of *Z. ramigera* ATCC 19544, with little or no reaction taking place with probes designed for *Z. ramigera* ATCC 19623 or *Z. ramigera* ATCC 25935 (Rosselló-Mora et al., 1995).

At least 90–100% of *Zoogloea* strains denitrify, demonstrate positive oxidase and catalase reactions, hydrolyze gelatin, carry out *meta* cleavage of aromatic ring structures, and fail to form acid from carbohydrates (Unz and Dondero, 1967a; Unz and Farrah, 1972). Approximately 82% of strains demonstrate urease activity. To define the salient characteristics of the genus is difficult, because minor attributes, such as acid formation from ethanol and glycerol, are often located in strains of a common natural provenance—i.e., progeny of a single zoogloea or of several zoogloea found in a specific wastewater treatment plant or polluted environment.

Floc formation (Finstein, 1967) and major exopolymer pro-duction (Unz and Farrah, 1976a) by *Zoogloea* strains take place during the decreasing and stationary growth phases. Consequently, bioflocculation and exopolymer formation are to be regarded as conditional characters of *Zoogloea* strains, albeit ones that are exhibited reliably by freshly-isolated organisms.

Degradation of the benzenoid ring structure by a *meta* cleavage mechanism is a distinctive character of *Zoogloea* strains and is considered taxonomically significant for the acidovorans group of *Pseudomonas* (Stanier et al., 1966). *Z. ramigera* differs from *P. acidovorans* and *P. testosteroni* by its ability to hydrolyze gelatin and to denitrify and by its inability to hydrolyze starch or to utilize glycolate, caproate, and several amino acids.

FURTHER READING

Zvirbulis, E. and H.D. Hatt.. 1967. Status of the generic name *Zoogloea* and its species. Int. J. Syst. Bacteriol. *17*: 11–21.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *ZOOGLOEA*

Because of the taxonomic difficulties described previously, the characteristics of the neotype strain of *Z. ramigera* are presented for comparison with those of two other strains whose inclusion in the species is considered by this author to be unwarranted (Table BXII.β.120). See also the section on Other Organisms.

List of species of the genus Zoogloea

1. **Zoogloea ramigera** Itzigsohn 1868, 30[AL]

   ra.mi′ ge.ra. L. n. *ramus* branch; L. v. *gero* to bear; M.L. adj.*ramigera* branch-bearing.

   The morphological, cultural, physiological, and nutritional characters are given in the description of the genus and in the description of the neotype strain (Table BXII.β.120). No growth occurs on Koser's citrate. H₂S is not produced from cysteine or on Kligler iron agar. Indole is not produced. Ammonia is produced from asparagine. Lipolytic activity occurs.

   Growth on benzoate, *m*-toluate, and *o*-cresol occurs with *meta* cleavage of the aromatic ring.

   Methanol, formate, and formaldehyde are not utilized as sole carbon sources.

   This strain was cultivated from a single cell isolated by micromanipulation of a finger-like zoogloea present in a trickling filter sample collected in Freehold, New Jersey.

   The mol% G + C of the DNA is: 68.9 (HPLC).

   Type strain: 106 (neotype), ATCC 19544.

   GenBank accession number (16S rRNA): X74913.

2. **Zoogloea resiniphila** Mohn, Wilson, Bicho and Moore 1999b, 935[VP] (Effective publication: Mohn, Wilson, Bicho and Moore 1999a, 76.)

   re.si.ni′ phi.la. L. fem. n. *resina* resin; Gr. adj. *philos* loving, friendly to; M.L. fem. n. *resiniphila* loving resins.

   Gram-negative, nonsporeforming rods. Catalase negative and oxidase positive. Aerobic, using nitrate, but not fermentative. Mesophilic, growing at 45°C but not at 50°C; however, also reported to grow at 30°C and 37°C, but not at 40°C (Mohn, 1995). Cells grown on dehydroabietic acid are 1.5–2.8 × 1 µm and motile, forming clumps bound by exopolymer; colonies are white, smooth, punctiform, and convex, tenaciously stick to agar.

   In mineral medium, use abietic, dehydroabietic, palmitic, benzoic, acetic, and pyruvic acids, as well as D-xylose, phenol, ethanol, and β-sitosterol. Use poorly 12- and 14-chlorodehydroabietic acids. Do not use pimaric, isopimaric, or linoleic acids.

   Main cellular fatty acids are $C_{16:1 \, \omega 7c}$, $C_{16:0}$, and $C_{18:1}$.

   The 16S rDNA sequences (EMBL AJ011506) groups within the class *Betaproteobacteria*.

   Isolated from a laboratory sequencing batch reactor in Vancouver, British Columbia, Canada.

   The mol% G + C of the DNA is: not determined.

   Type strain: DhA-35, ATCC 700687.

Other Organisms

In addition to the neotype strain of *Z. ramigera*, there exist two other strains that have attained some prominence through experimental use: strain 1-16-M (Crabtree and McCoy, 1967) and strain 115 (Friedman and Dugan, 1968). The three strains all share the property of floc formation, and each was obtained from polluted environments; however, phenotypic (Table BXII.β.120) and phylogenetic (Fig. BXII.β.117) dissimilarities among the strains indicate that strains 1-16-M and 115 should not be included in the genus *Zoogloea*. Additional evidence for this view is that cross-reactions are not observed between strains 1-16-M

TABLE BXII.β.120. Characteristics of *Zoogloea ramigera* ATCC 19544 and two misidentified *Zoogloea ramigera* strains of historical importance[a]

Characteristics	ATCC 19544	ATCC 19623	ATCC 25935[b]
Cell diameter is 1.0 μm or slightly larger	+	−	−
Flagellar arrangement:			
Monotrichous only	+	−	+
Monotrichous and polytrichous (lateral)	−	+	−
Zoogloeae are produced	+	−[c]	+
Straw-colored colonies	−	+	+
Growth on potato	−	+	
Hydrolysis of gelatin and casein	+	−	+
Tyrosine agar cleared	−	+	
Arginine dihydrolase	−	+	
Sensitivity to 0/129[d]	+	−[c]	
Growth in presence of 3% NaCl	−	+	
Litmus milk:			
Alkalinity produced	−	+	+
Reduction occurs	−	+	−
Denitrification (to N_2)	+	−	−
Urease activity	+	−	+
Hydrolysis of starch	−	−	+
Acid formed oxidatively from:			
Arabinose, fructose, sucrose	−	+[c]	+
Galactose, maltose	−	+[e]	+
Cellobiose, glycogen, lactose, mannose	−	−[e]	+
Glucose	−	+[c]	+[f]
Rhamnose, mannitol		+[c]	−[f]
Ribose	−	+[c]	
Utilized as sole carbon source:			
Acetate	+	+	+
Citrate	+	−	+[g]
Malate, pyruvate, fumarate	+	+	
Butyrate, α-ketoglutarate, propionate	+		+[g]
n-Propanol, ethanol, *n*-butanol	+	+[e]	+
Acetaldehyde, succinate, oxalacetate, lactate, α-hydroxybutyrate, palmitate, myristate	+		
Benzoate, *m*-toluate, *p*-toluate, phenol, *o*-cresol, *m*-cresol, *p*-cresol	+[h]	−[h]	−[i]
Methanol	−	+[e]	−
Mol% G + C of DNA[j]	68.9	63.6	63.4
Ubiquinone-10[k]	−	+	−
Ubiquinone-8[k]	+	−	+
Rhodoquinone-8[k]	+	−	−
Spermadine[l]	−	+	−
Putrescine[l]	+	−	+
2-Hydroxyputrescine[l]	+	−	+
3-Hydroxydecanoic acid[k]	+	−	+
3-Hydroxylauric acid[k]	+	−	−
3-Hydroxymyristic acid[k]	−	+	+
3-Hydroxypalmitic acid[k]	−	+	−
Palmitoleic acid[k]	+	trace	+

[a]Unless otherwise indicated, the sources of data for strains are as follows: ATCC 19544 (strain 106), Unz (1971); ATCC 19623 (strain I-16-M), Crabtree and McCoy (1967); and ATCC 25935 (strain 115), Friedman and Dugan (1968). For symbols see standard definitions.

[b]Proposed as *Duganella zoogloeoides* (Hiraishi et al., 1997b).

[c]Data from Unz (1971).

[d]Vibriostatic agent 0/129 is 2,4-amino-6,7-diisopropylpteridine.

[e]Data from Friedman and Dugan (1968).

[f]Data from Hiraishi et al. (1997b).

[g]Data from Joyce and Dugan (1970).

[h]Data from Unz and Farrah (1972).

[i]Only benzoate tested.

[j]Data from Shin et al. (1993).

[k]Data from Hiraishi et al. (1992c).

[l]Data from Hamana and Matsuzaki (1993).

or 115 and fluorescein-labeled, whole-cell antiserum against the neotype strain (Farrah and Unz, 1975). Weak, whole-cell antigen–antibody reactions do occur between strain 115 and strain 1-16-M, although strain 115 bears a greater antigenic relationship to *Gluconobacter oxydans* subsp. *suboxydans* (ATCC strain 621) (Chorpenning et al., 1978).

Recently, based on 16S rDNA sequence analysis, strain 115 (*Z. ramigera* ATCC 25935) has been proposed as the type strain for

the renamed type species, *Duganella zoogloeoides*, sp. nov., of a proposed genus, *Duganella* gen. nov. (Hiraishi et al., 1997b).

Nonextant strains of *Z. ramigera*, which bear some resemblance to the neotype strain according to published descriptions, are strain Z-1 (Butterfield, 1935) and an early isolate of questionable purity (Bloch, 1918).

Class IV. **Deltaproteobacteria** *class nov.*

JAN KUEVER, FRED A. RAINEY AND FRIEDRICH WIDDEL

Del.ta.pro.te.o.bac.te′ri.a. Gr. n. *delta* name of fourth letter of Greek alphabet; Gr. n. *Proteus* ocean god able to change shape; Gr. n. *bakterion* a small rod; M.L. fem. pl. n. *Deltaproteobacteria* class of bacteria having 16S rRNA gene sequences related to those of the members of the order *Myxococcales*.

The class is defined solely based on sequence similarity of 16S rRNA. It comprises several bacterial groups that had been previously treated as separate systematic assemblages according to phenotypic characteristics. 16S rRNA sequence analyses are not only informative for the definition of orders and families within the *Deltaproteobacteria*. This method is usually also relevant for the establishment of genera and sometimes species; nevertheless, phenotypic features such as nutritional characteristics or chemotaxonomic properties may be equally important at the genus level, and, in combination with DNA–DNA hybridization, at the species level.

The class *Deltaproteobacteria* comprises morphologically diverse, Gram-negative, nonsporeforming bacteria that exhibit either anaerobic or aerobic growth. Members with facultatively anaerobic/aerobic growth are not known so far.

Most anaerobic members can use inorganic electron acceptors that allow energy conservation by anaerobic respiration. By performing such reactions, these bacteria play a major role in the global cycling of elements. Utilization of inorganic electron ac-

ceptors is an important physiological and taxonomic characteristic. However, in a number of isolates the reduction of some electron acceptors (e.g., sulfur, ferric iron) may not be associated with growth (as in the case of oxygen reduction). Therefore, the utilization of an electron acceptor (especially in sulfate-reducing bacteria) must be confirmed by definite growth in subcultures. This has not been proven in every case.

Some anaerobic members are fermentative and/or exhibit syntrophic growth by proton reduction and interspecies hydrogen transfer.

One striking feature of the aerobic representatives is the ability to digest other bacteria. Several of these members are important constituents of the microflora in soil and waters.

Orders *Desulfurellales, Desulfovibrionales, Desulfobacterales, Desulfarcales, Desulfuromonales, Syntrophobacterales.*

Orders *Myxococcales* and *Bdellovibrionales* are exclusively aerobic.

Type order: **Myxococcales** Tchan, Pochon and Prévot 1948, 398.

Order I. **Desulfurellales** *ord. nov.*

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De.sul.fu.rel.la′ les. M.L. fem. n. *Desulfurella* type genus of the order; *-ales* ending to denote an order; M.L. fem. pl. n. *Desulfurellales* the order of *Desulfurellaceae*.

Cells are rod-shaped and usually motile. Strictly anaerobic chemoorganotrophs or chemolithoautotrophs with a respiratory type of metabolism; in addition, limited fermentative capacities may occur in some members. The common electron acceptor is elemental sulfur (or polysulfide), which is reduced to sulfide; thiosulfate may be used. Sulfate does not serve as electron acceptor. Simple organic compounds serve as electron donors and carbon sources that are completely oxidized to CO_2. So far as known, the mechanism of terminal oxidation is the citric acid cycle. All members are moderate thermophiles with temperature optima between 50 and 60°C. A striking biochemical feature in

species investigated so far is the absence of cytochromes, despite a respiratory metabolism. Menaquinones are present. Species have been isolated from geothermally heated, sulfidic freshwater and marine environments. The order currently contains only one family, *Desulfurellaceae*.

Type genus: **Desulfurella** Bonch-Osmolovskaya, Sokolova, Kostrikina, and Zavarzin 1993, 624 emend. Miroshnichenko, Rainey, Hippe, Chernyh, Kostrikina and Bonch-Osmolovskaya 1998, 478 (Effective publication: Bonch-Osmolovskaya, Sokolova, Kostrikina, and Zavarzin 1990, 155.)